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BEHAVIOUR OF *AEDES* MOSQUITOS IN RELATION TO BLOOD-FEEDING AND REPELLENTS

BY

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About four thousand females of *Aedes aegypti* (L.) and two thousand of several Canadian species of *Aedes* were studied in the field and the laboratory. Their behaviour in relation to a host and to repellent chemicals was divided into eight phases and the conditions influencing them as well as the stimuli responsible for the initiation, inhibition, and termination of these phases were investigated; orientation was also considered. The factors studied include surface temperature, convective and radiant heat, light and colour, moisture, wind, odour, taste, gravitation, sound, and movement. The relevance of these results for the development of improved mosquito repellent chemicals is discussed.

The blood-feeding behaviour of *Aedes* mosquitos differs somewhat between species and is influenced by, among other things, age, virginity, the number of blood meals, and the external conditions. An attempt was made to subdivide the total behaviour leading to biting into recognizable successive phases and to describe these. Some attention was also paid to the possibility that the complete normal behaviour sequence was not in all circumstances necessary for a successful blood meal but that various activities could lead up to this result. Table I summarizes the information obtained concerning these phases of behaviour in their normal sequence and indicates the experiments from which this information was derived. Each phase of behaviour is first described and then discussed where applicable in respect to the general internal and external conditions, the initiating and facilitating stimuli and their interactions, the orientating stimuli, and finally in relation to

TABLE I

Information concerning behaviour phases and stimuli associated with them

Behaviour phase	General conditions Internal & External	Initiating and facilitating stimuli	Orientating stimuli	Quietening and inhibiting stimuli
1. *Rest:*	2, 3, 4*	2, 8	1	4, 12
2. *Flight:*	2, 4	2, 4, 12	5, 9, 11	12
3. *Spontaneous exploratory behaviour:*	2, 7; 8	2, 7	2	—
4. *Searching on the wing:*	2, 4, 6	2, 4, 5, 6, 7	2, 5, 7	7, 8
5. *Settling on host, (cf. rest):*	5, 6, 7, 8, 9	5, 6, 8	1, 7, 8,	12
6. *Searching by walking:*	10, 11	10, 11	1, 5, 10, 11	12
7. *Probing and biting:*	4, 10, 11	5	5	12
8. *Taking off:*	12	12	—	12

* Numbers of appendices describing experiments from which the information was obtained.

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quietening and inhibiting stimuli. As most experiments yielded information on several of the above points, they are described separately afterwards.

It follows that many of the individual tests reported are in the nature of partial replications. Some tests involving small numbers have been omitted.

RESTING BEHAVIOUR

In nature female mosquitos appear to spend most of the time sitting head upwards on vertical surfaces of suitable texture, under cover, and where the saturation deficiency and light intensity are rather low.

Very little is known of the effect of age on the extent of rest or of the variation between species. Our data indicate that younger individuals are more easily aroused both mechanically and by the visual perception of movement. Exhaustion of carbohydrate food reserves and engorgement with blood are both conducive to rest. Effects of diurnal changes in light, temperature, humidity, and other elements of weather on movement and lack of movement have been described for several species (HOCKING, RICHARDS & TWINN, 1950; CURTIS, 1953; NIELSEN & NIELSEN, 1953; PLATT, COLLINS & WITHERSPOON, 1957; PROVOST, 1952; SNOW & PICKARD, 1957).

Our observations show that in complete darkness, *Aedes aegypti* (L.) flies probably only when mechanically aroused. But mating is normally initiated while on the wing, and this species can be reared in total darkness (JOBLING, 1937). In the dark, certainly, more intensive stimulation is required for flight. At very strong light intensities, exceeding normal conditions in the resting habitat, mosquitos will also rest more than at moderate intensities (appendix 2, p. 8). At low temperatures most mosquitos rest. Gravitation is apparently the stimulus responsible for the head upward orientation (appendix 1, p. 8).

FLIGHT BEHAVIOUR

Next to rest mosquitos probably spend the greatest amount of time in flight. Except in the circumstances to be described, walking is apparently rare. Flight forms part of many activities, such as swarming, mating, oviposition, and migration, so that only part of the time spent by a female on the wing is in the service of the search for blood. It is difficult to observe in nature what a flying mosquito is up to.

In many species the diurnal activity patterns of flight are bimodal and crepuscular. Little is known about the inner clock but there is a striking dependence on light intensity; the times of most flight move with the times of sunrise and sunset, are more prolonged on overcast days and shorter on clear days (HADDOW, 1945; MONCHADSKII, 1950). The availability of nectar limits the duration and range of active flight (HOCKING, 1953). If suitable sources are available mosquitos may travel great distances, especially when wind borne.

In general, stimuli which facilitate rest inhibit flight and vice versa. The results given in Table II suggest that either strong wind or completely still air tend to inhibit light, but that moderate wind may induce flight, often of an orientated nature. Illumination, apart from very low and very high intensities, is favourable to flight activity, and for *A. aegypti* the optimum conditions seem to be a wind speed of about 15 cm/sec (0.5 km/hr, or 0.3 mph), a wind velocity only just perceptible to man, and a light intensity of 2—3 metre candles (5—10 foot candles).

Mechanical disturbance and the visual perception of movement provoke flight activity (appendices 2 and 4, p. 8—12). Acoustic stimuli, of importance in the mating behaviour of the male (KAHN, 1947; ROTH, 1948; WISHART & RIORDAN, 1959), could not be shown to induce flight (appendix 3, p. 10) or any other response in female *A. aegypti.* Human body odour or the scent from a guinea pig added to an air stream rapidly increased the numbers of mosquitos flying in a cage to a remarkable extent (appendix 5, p. 12). A similar effect was obtained with repellent odours (cf. KENNEDY, 1939). This interaction between wind and odour has been further studied by BÄSSLER (1958).

Much of the flight of a mosquito seems to be controlled by optomotor reactions. In particular it can be shown that flight against an air stream, whether aroused by chemical or thermal stimuli or not, depends on the visual perception of relative movement. In most conditions mosquitos will move against the airstream — i.e. they maintain a constant relationship with a visual pattern, or even overtake it against the effects of the airstream. Within suitable limits they will thus land on screening covering the air inlet into a cage. It was shown that this reaction ceases at low light intensities, which however, still permit unorientated flight. Table III (appendix 2, p. 10) suggests that at light intensities below 0.2 ft candles and with no special provision of visual landmarks, between 2 and 7 seconds are required for orientation into the wind.

As in most insects, tarsal contact will inhibit flight, except in the special circumstances described later.

SPONTANEOUS EXPLORATORY BEHAVIOUR

Open air observations with binoculars (appendix 7, p. 14) did not show any spontaneous searching at dusk at a time when many mosquitos were biting. A difficulty in the investigation of spontaneous movement is the inevitable disturbance caused by the presence of the observer who is also the target for the female mosquito. Even if flying mosquitos are observed from a distance, or by special means such as in a cage in a dark room, it is difficult to be sure that their activity is one of search and that they have not been aroused by the observer.

Data on flight activity in relation to light and wind (appendix 2, p. 8) appear to us to apply to true searching flight, but this may not be so.

Female mosquitos, even when they are flying spontaneously, apparently orientate mostly into the wind.

SEARCHING ON THE WING

Some of the difficulties mentioned in the discussion on exploratory behaviour apply also to provoked searching behaviour. But there is no doubt that this behaviour, released by external stimuli, occurs. It is unmistakable when one watches a mosquito approaching oneself in nature, or approaching a guinea pig in a cage.

Interest of females in stimuli provoking searching is low at time of emergence and increases at varying speeds in different species. A temperature-time product probably determines the onset and termination of this behaviour, but appendix 4 supports the periodicity of about 5 days found by LAVOIPIERRE (1958) and BURGESS (*in litt.* 1959). Short-term conditioning occurs (appendix 6, p. 14), with the effect that mosquitos at rest in a suitable state respond more readily by searching for the source of partial stimulation such as heat or odour a few minutes after complete stimulation from a living host than they do before this. There is some indication that with man as the host this conditioning may be individual-specific. Nothing is known about long term conditioning effects. External conditions favouring searching on the wing appear to be the same as those favouring general flight activity.

The transition from rest to searching probably occurs in several situations. Any sensory receptors may contribute to this transition. Combinations of sensations are particularly important and stimuli resembling those provided in proximity to a host are a profitable starting point for investigations. For instance, a finger tip placed 1 cm below a female mosquito on a wall will arouse it to flight (appendix 5, p. 12).

Several stimuli affect the orientation of mosquitos on the wing. Mosquitos will fly up-wind if the wind speed does not exceed the cruising flight speed and when sufficient visual orientation is possible, i.e. if the environment contains distinctive mosquito-visible features and the illumination is adequate (appendices 2, 5, 7, p. 8—14). Many mosquitos settle on an up-wind screen when human odour is provided (appendix 5). Searching on the wing in the open is a more complex form of behaviour than just flying into a wind. Mosquitos may overshoot an objective and come back, indicating pilotage of some kind, but we know little about this. The orientation of movement in the field by temperature and possibly humidity is determined by differentials of these quantities rather than absolute values. Extreme absolute values, however, may inhibit particular reactions entirely (appendix 8, p. 16).

Factors inhibiting searching are little understood. Probably fatigue and previous failure to find a host are among the conditions which prevent female mosquitos in an otherwise suitable physiological state from searching continuously. In cage experiments searching ceases fairly quickly unless its object, the blood meal, is obtained. The number of mosquitos searching for a host is a general function of flight activity, since a flying mosquito is more readily attracted than a sitting one. Searching on the wing passes directly into searching by walking when a host is found and settled on. In a poplar wood it was observed that mosquitos approached people from down-wind and at ankle height, then settled and moved by walking upwards on the down-wind side of the person. Lying down and keeping still in a meadow for a few minutes resulted in the dispersal of most mosquitos, while they were able to follow a person at any running speeds of which he was capable, either in the open or in woods. Field observations on the approach to the host agree with laboratory observations on behaviour in relation to obstacles in an air stream (appendix 7, p. 14). Mosquitos nearly always settled on the down-wind side of such an obstacle. The fact that mosquitos could land on the down-wind side of a bluff body when the wind speed was considerably higher than that which prevented them from landing on a streamlined body suggests that they may be guided by a velocity gradient.

SETTLING ON HOST

Studies of settling behaviour were conducted with heatable polyethylene bottles covered with cloth, styrofoam models, and with actual hosts (man, dog, guinea pig) and are described in appendices 5, 6, 8, and 10 (p. 12—20).

Certain textures of surfaces seemed to be favourable for settling and resting; for instance, wool jersey, wood, and stryrofoam. Smooth surfaces such as cellulose acetate sheet, glass, and surfaces coated with polytetrafluor ethylene ("Fluon", MERTON, 1956) were progressively less favourable to settling, in this order. Sometimes other authors' findings that dark shades were more attractive than light ones (GJULLIN, 1947; BROWN, 1951) could be confirmed, but at other times we got abundant settling on white surfaces. A very attractive substrate was red wool jersey but we do not know whether mosquitos distinguished this substrate by the characteristics of its reflected radiation, nor whether the receptors responsible are the eyes. It is unlikely that general light reflectivity, thermal radiation, surface temperature, or chemistry are responsible as more mosquitos landed on several red materials than on white, black, or some greys, over a wide range of both light and temperature. Fig. 3 (p. 18) shows the colour reflectance of the red wool jersey to be rather closely complementary to that of green vegetation; this may be significant and implies a measure of colour sensivity. The relationship between size of polyethylene bottles and the number of mosquitos landing on them suggests that visual and thermal factors are insufficient to explain attraction to these dummies.

Through most of the physiological temperature range more mosquitos were found settled on warm surfaces, approximating blood temperature, than on cold surfaces (appendices 8, 10, p. 16—20); but we do not know whether this is due to more mosquitos settling or to mosquitos resting longer.

At very high ambient temperatures and humidities, e.g. 37.8° C (100° F) and 100% R.H., no orientated flight was observed and biting could not be obtained (cf. DELONG, 1946; BROWN, SARKARIA & THOMPSON, 1951) (appendix 8). In the field on the other hand, in misty conditions directly after a hail storm *Aedes fitchii* (Felt and Young) and *A. stimulans* (Wlk.) alerted by mechanical disturbance, pursued the observers, settled on them, and bit.

Large numbers of *A. aegypti* settled on worn nylon or wool socks even in the absence of a temperature gradient; presumably they were orientated by odour. Socks kept for three weeks in sealed containers retained some of this attractiveness (appendix 5). Our findings in respect of odour agree well with those of BROUWER (1958), HADDOW (1942), LAARMAN (1955) and RAHM (1956, 1957, 1958a, 1958b).

Attraction of *A. aegypti* by host odour seems to be favoured by higher wind speed and lower light intensity than is spontaneous flight.

SEARCHING BY WALKING

After settling a mosquito either reverts to a state which looks like rest, i.e. it remains immobile, or it takes off again, or it starts searching by walking. Searching movements are characteristic and somewhat bizarre, reminiscent of movements in complex optomotor situations; e.g. walking backwards or sideways, and turning even into a head down position. Searching by walking is very often accompanied by probing. The behaviour seems to be controlled by a combination of surface temperature gradients, perhaps by the slight local air currents resulting from these, and by odour or contact chemical stimuli. Mosquitos frequently alight in a low position on an attractive object in wind, i.e. on man, a guinea pig, or a warm bottle. This is usually followed by walking upwards (appendices 10, 12, p. 20). Downwards walking is comparatively rare and is nearly always accomplished by walking backwards or sideways; walking forwards with the head down is very rarely seen. Having alighted above a host or substitute for a host the mosquito usually takes off and returns on the wing lower down (appendix 10).

PROBING AND BITING

Probing behaviour consists of two parts; transverse swinging of the body about a dorso-ventral axis to both sides alternately and a rhythmical raising and lowering of the head and proboscis to bring the tip of the latter in contact with the substrate.

Probing occurs either immediately on landing, during a searching walk, or after its termination. It appears to be induced by warmth or odour and if not terminated by biting is persisted in for some time. It is often visibly orientated against a suitable object such as a finger tip or a warm test tube. It is also sometimes deflected away from a repellent source, e.g. a cold object, such as ice, as is also searching by walking.

The transition from probing to biting is difficult to observe with the unaided eye. It may be immediate or may take a few seconds. *A. aegypti* females continue to feed for three to five minutes if undisturbed and once the process has started it is difficult to interrupt. For instance a mere trace of smoke from burning cardboard in a bee smoker causes sitting mosquitos to take off and flying mosquitos to evade; a biting mosquito will continue to feed in quite thick smoke.

Presumably biting is released by a proper consistency of the substrate and by chemical stimuli resulting from successful probing, i.e. from touch by the proboscis. Probing cannot be induced directly after a blood meal.

TAKING OFF

This is normally released by the insect being filled, but some very replete mosquitos were reluctant to take off. Premature taking off can only be obtained as a result of very strong stimuli (appendix 12, p. 21).

BEHAVIOUR IN RELATION TO SO-CALLED REPELLENT CHEMICALS

As DETHIER (1956) has pointed out attraction and repulsion, which imply orientated reactions, should be distinguished from circumstances which keep a mosquito settled or at rest or which make it take off or fly away. In practical experimentation this distinction is often difficult to make. The apparent repellency of attractants sometimes reported is often a question of concentration (DETHIER, 1947). Other anomalous results may be explained by the different effects which chemical stimuli may have on the various phases of behaviour described above. Irritant vapour may make resting mosquitos take off and thus increase the numbers of animals which can respond to a landing test being applied. Acting at a later phase the same chemical may, by true repulsion, reduce the numbers landing on the screen used in the test. In a laboratory test of repellency of the usual type, e.g. putting a forearm in a cage, the total result will depend on the relative magnitude of a number of effects; in other circumstances, in particular in the open, quite a different end result may be observed. One has the impression that one and the same chemical may both facilitate and inhibit various phases of locomotion, resulting in peculiar combinations of behaviour. Mostly, one phase of behaviour is stimulated while another is inhibited. The search by a mosquito may be maintained by the warmth and odour of an exposed part of the body, while settling and probing are prevented by a repellent chemical. Thus if we count mosquitos biting or probing the repellency in the situation becomes apparent, while if we count numbers approaching or even settling nearby, attraction might be demonstrated.

The ultimate goal in applying chemical repellents should be to keep the insects at a comfortable distance so that they do not bite, probe, settle, or search in the immediate neighbourhood of people. It would thus appear that a repellent which would prevent searching would in theory be the most desirable. Effects of this nature have in fact been observed in experiments using repellent loaded air streams. When we covered half of the air entry screen of the wind tunnel cage (appendix 2, p. 8) with a second screen coated with dimethyl phthalate, the other half serving as control, fewer mosquitos settled on the treated half, (appendix 12, p. 21). This is a field where experiments with olfactometers may be relevant. Tests with restricted areas of the hand treated with repellents and the hand placed immediately below a small glass tube containing repellents tend to confirm this indication of action through the olfactory sense.

Some attractive factors clearly operate even in the presence of a repellent (appendix 12). The chemical attractive factors may be partially neutralized by the repellent, leaving thermal and visual factors in operation to draw a small number of mosquitos. The masking of an attractant odour by a repellent (RUDOLFS, 1923) appears to us unlikely in the light of VON FRISCH's (1919) findings. On the other hand contact between repellent chemicals as liquids and substantial areas of the proboscis, tarsi and tibiae, mesonotum, or the wings leads to the interruption of biting, and in mosquitos not engaged in biting to the retraction of the touched limb or limbs or to take off. This would appear to be due to a stimulation of the general chemical sense rather than the sense of taste.

The failure of the inhibition of flight by tarsal contact when the surface is coated with repellent, may represent an interference with the function of the tactile receptors or again be an effect through the little understood general chemical sense. PETERS & KEMPER (1958) have indicated their belief that repellents act through the general chemical sense; their description of this as a "crude disturbance of the normal physiological state" of the integument, suggests that it may be attributable to the same qualities in the repellent chemicals that make them, almost without exception, good plasticisers.

The term gustatory repellent has often been used with reference to recently developed materials; ammonium chloride has been claimed to have this property towards *A. aegypti* by FRINGS & HAMRUM (1950) and towards *Periplaneta* by BLOCK (1951). We were unable to demonstrate effects of this nature with any of the following materials applied on the skin, although many of them are known to cancel the acceptance stimuli of sugar solutions to the other insects: quinine hydrochloride, aminophylline, urea, soluble saccharine (Na salt), extract of quassia, aloes, colocynthin, asafoetida. This leads us to suppose that rejection is not mediated by chemoreceptors on the labella of these mosquitos; there may, however, be some inside the labrum-epipharynx or buccal cavity (c.f. MILES, 1958; DAY, 1954). If this be so, there is hope for the development of a systemic gustatory repellent, either oral or intravenous (KINGSCOTE, 1958); although valueless against nuisance species, this would be useful against disease vectors.

Two and perhaps three attributes, then, seem to be required in a mosquito repellent: a repulsive odour, opposing the attraction of host odours; a contact irritant action through the general chemical sense; and true gustatory repellency. A logical procedure in the orderly development of better materials would seem to be to measure each of these attributes separately (CHRISTOPHERS, 1947) for materials known to be useful, when meaningful correlations with chemical structure or physical properties may be anticipated. This in turn should permit the development of better materials for each attribute separately and mixtures of materials may then perhaps be confidently formulated.

APPENDIX 1

Centrifugation. A 9 cm petri-dish was lined at the bottom with filter paper with one radius drawn on it in pencil; the inside vertical face was coated with Fluon. Ten *A. aegypti*, males and females, were placed in the dish which was then covered with cellophane and attached to the head of a centrifuge. The dish was observed at 390 r.p.m. by stroboscopic illumination; mosquitos faced towards the centre of he dish, and sometimes walked towards it. The average centrifugal force under these conditions was about 1 g.

On a vertical turntable rotating once in 20 seconds about a horizontal axis, female *A. aegypti* will show counter-rotation to maintain a head upward position.

APPENDIX 2

Effect of light and wind on flight activity. Between 300 and 500 *A. aegypti* females of mixed age were held in a cage 60 cm high by 90 cm by 60 cm. The bottom and ends were of plywood, the top and sides of glass. A 15 cm diameter centrifugal blower driven by a ¼ H.P. motor with variable transformer speed control was arranged to draw air from a 15 cm diameter hole in one end with 1 mm mesh saran screen covering. Air was admitted through a 15 cm screened hole opposite this in the other end. This resulted in a fairly discrete cylinder of wind in the centre of the cage, with relatively less air movement elsewhere. A white card with squares ruled on it in pencil was placed behind the cage to facilitate observation. The cage and the card were illuminated by two tubular 60 watt filament lamps one in each of the front vertical corners, also controlled by a variable transformer (Fig. 1). Water and soaked raisins were constantly available to the mosquitos.

Illumination and wind speed were set at various combinations of intensity and the cage was left undisturbed for periods of at least an hour, after which a quick estimate of flight activity was made, as far as possible without introducing any disturbing factors. Temperature varied with illumination and wind, but was recorded. At selected wind speeds and illuminations, the observers hand was placed in the air current, a few inches upwind of the inlet screen, and the rate of increase of mosquitos settled on the screen was noted. The results are summarized in Table II.

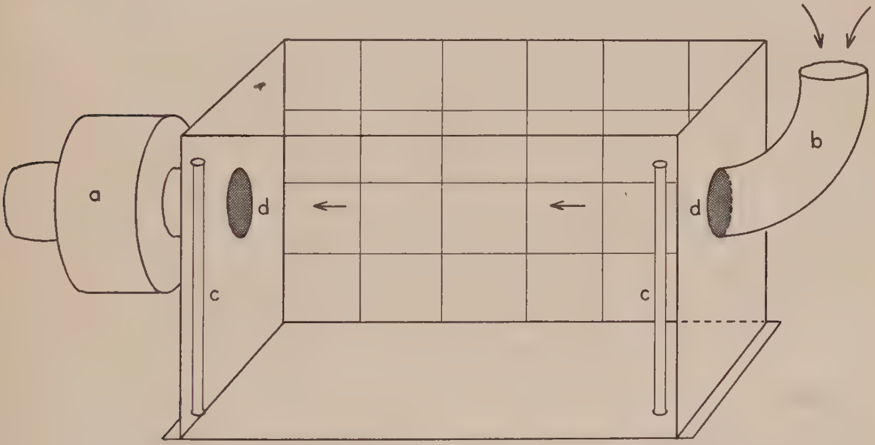

Fig. 1. The wind tunnel cage described in appendix 2. a, centrifugal blower; b, elbowed inlet; c, lamps; 2, 1 mm mesh screens.

In a further experiment, the numbers of mosquitos settled on the air inlet screen and on the air outlet screen were counted at various combinations of light intensity and wind speed. The results are shown in Table III. The large numbers of mosquitos which settled on the upwind screen led us to suspect the odour of the observers as a factor in this, but neither drawing the air through activated charcoal nor taking it direct from an outside source affected this.

To decide whether *A. aegypti* would fly in total darkness a cylindrical glass jar 8 cm diameter by 20 cm high was painted with Fluon inside for a distance of 5 cm down from the top. This was then inverted over a similar but unpainted jar containing 10 mosquitos, covered by a celluloid sheet. The assembly was

TABLE II

Flight activity of A. aegypti *under various conditions of wind speed and illumination*

Wind speed cm/sec.	Light ft candles	Temp. °F	Approx. No. Flying	Comments Activation by hand odour
0	0	78	0	—
	0	78	0	—
	0	81	0	—
	<0.2	79	2—3	—
	0.4	83	8—10	—
	1.6	81	8—10	—
	9	91	15—20	—
	12	95	6—8	—
	25	100	50—60?	Different age composition
13	0	79	20	—
	0	71	10—20	—
	0	71	12—24	—
	0.4	77	50—60	16 on screen to 85 in 1 min.
	0.4	77	50—60	11 on screen to 46 in 40 sec.
	0.4	—	30	30—75 in 1 min.
	1.6	78	30—40	—
	1.6	77	65	20—25 in 1 min.
	9.0	79	80	—
	25	80	20—30	—
23	0	80	0	0—60 in 1 min.
	<0.2	78	6—8	Total numbers low
	0.4	80	10—12	—
	5.3	81	20—25	—
	9.0	83	12—15	—
	25	78	8—10	Total numbers low
38	0	81	0—2	—
	<0.2	80	6—10	Rapid activation
	<0.2	82	6—8	Rapid activation
	1.6	79	10—20	1—150 in 1 min.
	9.0	82	15—20	Rapid activation
	25	79	4—6	—

placed in total darkness, the celluloid sheet was slipped out, and it was left for several hours. This was repeated several times; no mosquitos were ever found in the upper jar unless the jars had been moved around or subjected to some other mechanical disturbance.

APPENDIX 3

Acoustic stimuli. In view of the results of ROTH (1948), an audio-oscillator with frequency range from 0—20,000 cycles per second was placed alongside a cage of *A. aegypti* of assorted age. No reactions could at first be noted at any available intensity anywhere in the frequency range. Later mating reactions of males to flying females and a shuffling movement of the legs of sitting males accompanied by a subflexion of the abdomen were noted at frequencies between 250 and 550 cycles/sec.

TABLE III

The numbers of mosquitos settled on upwind and downwind screens at various wind speeds and light intensities; upwind above

Wind speed, cm/sec.	0	7.5	13	45	Totals	Ratio, upwind/downwind
Time for cage passage, sec.	∞	12	7	2		
Light Intensity						
6 volts*	5	1	1	1	8	0.04
	10	45	70	80+	205+	
		IV**	IV	IV		
10 volts	9	30	22	4	65	0.53
	5	17	42	58	122	
		I	II	IV		
20 volts	9	68	59	22	158	0.89
	7	24	47	100+	178+	
		IV		IV		
0.2 ft candles	23	56	66	44	189	1.4
	9	22	34	74	139	
	II	IV	III	III		
0.8 ft candles	14	68	61	54	197	1.7
	10	12	24	67	113	
		IV	IV			
4 ft candles	24	61	87	61	233	2.0
	17	22	32	46	117	
		IV	IV			
8 ft candles	5	41	49	35	130	1.1
	6	22	29	66	123	
		II	II	III		
25 ft candles	14	43	82	70+	209+	1.3
	5	23	28	100+	156+	
	I	II	IV	II		
Totals	103	368	427	291+	1189+	1.0
	69	187	306	591++	1153++	
Ratio, upwind/downwind	1.5	2.0	1.4	0.49	1.0	

* Voltage applied to two 60 watt filament lamps; illumination too low for measurement by equipment available.

** Significance of pair differences:
I $0.10 > P > 0.05$
II $0.05 > P > 0.01$
III $0.01 > P > 0.001$
IV $0.001 > P$

APPENDIX 4

The influence of age on avidity and response to visual and mechanical stimuli.

Three similar cylindrical cages 50 cms × 40 cm diameter made of acetate sheet with plywood ends were stocked with *A. aegypti* males and females which emerged within 3 separate 24 hour periods, July 21, July 23, and July 26. In one end of each cage was a 10 cm diameter hole closed by two flat nylon brushes with 6 cm bristles, the bristles facing each other over the hole. This permitted the arm to be inserted and withdrawn without loss of insects. The cages contained about 250, 130, and 225 mosquitos respectively. The cages were held at room temperature (23—26° C), humidity averaged 40% R.H., and water and soaked raisins were available in each of them. Responses to visual stimuli were gauged by estimating the proportion taking off in response to the rapid movement of the arm in a white coat sleeve, twice along the axis of the cage and close to it. Responses to mechanical stimuli were gauged by raising one end of the cage slowly 5 cm above the bench and then dropping it and estimating the proportion flying. Avidity was estimated by averaging the numbers biting the forearms of two different people exposed each for one minute inside each cage. Ready responses to visual and mechanical stimuli and the peak of spontaneous flying activity preceded the general peak of avidity by several days. The mean percentages biting at different ages from emergence was as follows:

Day	4	7	9	11	14	16
% Biting	27	61	50	69	13	16

APPENDIX 5

Olfactory stimuli. Without wind:

The numbers of *A. aegypti* landing on freshly washed socks and on similar socks which had been worn for 4 days were counted. The socks were placed in petri dishes on the floor of an observation cage with the worn and control items at diagonally opposite corners. The dishes were interchanged between successive counts. All handling was with tongs. Both beige nylon and grey wool socks were used. The number landing on the back of a hand freshly washed with detergent and rinsed in tepid water were also compared with those on an unwashed hand of the same person. The results are shown in Table IV.

TABLE IV

The numbers of A. aegypti *landing on washed and unwashed socks and hands in still air, at room temperature*

	Socks immediately after wearing	After 4 days in closed containers	After 18 days in closed containers	Hand BH	Hand HK
Washed	7	25	10 (3♂)	38	43
Worn or unwashed	38	45	18 (4♂)	56	50
P	between 0.01 and 0.001	between 0.10 and 0.05		about 0.2	

With wind:

A number of experiments were conducted on the effects of the combined stimuli of odour and air movement. The cage described in appendix 1 was used, but was fitted with a right angle bend of 15 cm diameter tubing with 30 cm radius, divided longitudinally into two halves, at the entry hole. Comparisons were made of the numbers of mosquitos settling on either half of the screen when sources of odour were placed in the pathway of either half of the entry tube. Most tests were repeated with left and right halves reversed; some also with a horizontal division and top and bottom halves reversed. Plain air was drawn through both halves for a few minutes between successive tests. Changes in numbers were brought about in part by the settling of new individuals, in part by mosquitos walking sideways across the screen. The results are summarized in Table V.

TABLE V

The numbers of A. aegypti *landing on screens through which wind borne odours are emitted*

	Left & Right	Reversed	Top & Bottom	Reversed	Totals	P
Grey wool socks (B.H.)						
Washed (left)	46	33	—	—	79	< 0.001
Worn 4 days	90	76	—	—	166	
Beige nylon socks (H.K.)						
Washed (left)	37	29	—	—	66	< 0.001
Worn 4 days	66	94	—	—	160	
Plain air (control)	55	69	73	38	252	about 0.5
Cotton cloth after 15 min. in armpit	63	57	61	62	233	
			Hand odour added to both sides			
100 dead *A. aegypti* ground up on wet paper towel.	10	9	18	16	53	about 0.6
Wet paper towel (control)	8	7	15	17	47	
Guinea pig	9, 14	13, 21	22, 27	18, 14	138	< 0.001
Plain air (control)	1, 2	9, 8	7, 8	3, 1	39	

Repellent effects of the dead bodies of the same species as reported by HEINTZ (1955) and SUDD (1957) could not be demonstrated.

An attempt was made to separate the effects of odour and of convective air currents (PARKER 1952, PETERSON & BROWN 1932) by using the back of the hand placed against the centre of the inlet screen of the cage as an attractive stimulus and placing a 4-bladed suction fan, 25 cm diameter, driven at about 45 r.p.m. by a stirrer motor, on the floor below this, to create a downward air current comparable in magnitude to a normal upward convection current. Only about half as many mosquitos landed with either normal or reversed convection currents as with a horizontal wind of about 20 cm/sec. When the normal upward convection was artificially supplemented, walking forwards with the head down and walking backwards with the head up were both observed.

Tests of worn socks and a guinea pig were also done with the T-tube apparatus

(Hocking & Lindsay, 1958); these gave olfactory response figures of +0.48 and +0.60 respectively.

When a finger is brought near a mosquito resting on a vertical wall, if it touches the wall about 1 cm below the mosquito, it will fly off after a few seconds and may return and settle on or below it or on the face, probe and bite. If the finger touches above, the mosquito will usually walk up and bite. If it touches at one side, after some delay the mosquito will turn, probe and bite.

No differences in attractiveness could be demonstrated between 4 hands which had just been heavily bitten and scratched (29 and 53 bites in 2 minutes), and the other hands of the same observers which had not (39 and 42 bites). Despite the differences in secretions and in odour the tip of the nose and the tip of the tongue incurred similar numbers of bites (about a dozen/minute) to the tip of the finger. Reactions to blood as such could not be demonstrated.

APPENDIX 6

Conditioning. During experiments with repellents (appendix 12, p. 21) it was noticed that many more mosquitos would land on a small area of skin (5 cm diameter) offered in a cage if the mosquitos had first been fully stimulated by waving the whole arm in the cage. There was some evidence that this conditioning was specific to the individual. The results of an attempt to confirm this are given in Table VI; they are inconclusive, but could only be expected to be otherwise if the conditioning was very short-term.

TABLE VI

Conditioning: The numbers of A. aegypti *landing on 5 cm circles on the backs of the hands of 4 individuals, otherwise covered with rubber gloves*

Whole arm exposed 2 min.; conditioning	5 cm circle exposed for 1 min.; 30 sec. intervals between tests.				Totals
H K 1358—1400 hrs.	N H(♀) 6*	H K 9	B H** 9	J H(♀) 4*	28
B H 1415—1417 hrs.	J H 10*	B H 16	N H 8*	H K 4	38
J H 1430—1432 hrs.	B H 22	J H 8	H K 11	N H 8*	49
N H 1445—1447 hrs.	H K 15	N H 10	J H 8	B H 14	47

* No previous conditioning, average number landing 7.2. Overall average number landing 10.1; on subjects to which previously conditioned, average number 11.4. Difference not significant.

** Mosquitos were previously conditioned to this subject about 1200 hrs.

APPENDIX 7

Wind and obstacles. Relations between atmospheric conditions and mosquito behaviour have been studied by Rudolfs (1923). Our field observations with *A. cataphylla* Dyar, *A. fitchii*, *A. excrucians* (Wlk.), and *A. stimulans* were facilitated by setting up a large vertical white plastic screen as a background and watching from the distance through binoculars. The behaviour of females of these species was observed in the vicinity of a host (man and dog) moving or standing in front of the screen and also in the absence of a host. Mosquitos nearly always land first on a stationary host low down on the downwind side. They then crawl or fly by hops upwards, probing at intervals. Movement of the host results in the assembly of more mosquitos partly by mechanical alerting from adjacent vegetation and partly by optomotor alerting from a greater distance; the position of landing is then determined by the relative wind rather than the true wind. If the host remains completely stationary, especially in a prone position, there is no assembly of more mosquitos, and many of those already assembled disperse.

This behaviour in relation to wind and obstacles was studied in the wind tunnel cage in the laboratory with *A. aegypti* using shapes made of styrofoam (foamed polystyrene which has a good surface for landings), stained deep red to improve its attractiveness. Various shapes were used; those which gave the most information were a flat cylinder 2.5 cm thick by 5 cm diameter, and a streamlined body of the same circular sectional area, and an overall length of 9 cm. The flat cylinder was used with its diameter horizontal when, in a wind, mosquitos concentrated on the curved surface within 30° each side of the downwind radius. It was also used with the diameter vertical, as a bluff body in comparison with the streamlined shape. Fig. 2 shows the typical distribution of mosquitos on the streamlined body in still air, and in a moderate wind. Table VII shows the numbers and distribution of mosquitos on both shapes at various wind speeds, starting with still air.

TABLE VII

The numbers of A. aegypti *remaining on a streamlined and on a bluff body at various wind speeds, and their distribution*

		Flat cylinder		Streamlined body	
Wind Speed cm/sec.	Upwind flat 19.5 cm^2	Curved surface 39 cm^2	Downwind flat 19.5 cm^2	c. 103 cm^2 No.	Distribution
0	14	14	16	38	Sub-uniform
13	0	1	12	20	None upwind of broadest section
36	2	1	4	14	None upwind of broadest section
60	1	0	5	6	All near downwind apex.
95	0	1	3	0	—
125	0	0	4	0	—
160	0	0	0	0	—

The overall differences between upwind and downwind position and between flat and streamlined bodies are statistically significant.

The maximum wind speed at which mosquitos were able to settle on the bluff body was 95 cm/sec., that for settling on the streamlined body was 55 cm/sec. Further confirmation of these observations was obtained by standing a polythene bottle of water heated electrically to 44.4° C (112° F) and covered with red wool jersey (see appendix 8) in the cage with its top half in the wind column between the screens and its bottom half below this. Mosquitos landed consistently all around the lower half, but only on the downwind side of the upper half.

APPENDIX 8

Studies on settling. Important aspects of this have been described by PARKER (1952), and PETERSON & BROWN (1951). Among other findings they report a failure in inducing mosquitoes to settle of warm dry objects, when convection currents were eliminated.

In order to study the influence of temperature and humidity of air streams on settling, a 15 cm diameter circular grid of nichrome wire was made to fit over the entrance to the wind tunnel test cage. To minimize any possible effects of radiant heat, this was subsequently placed horizontally on top of a stove pipe elbow leading to the inlet. The corrugations of the elbow reduced specular reflections. The grid was divided into two halves which could be heated independently or together, controlled by separate variable transformers. The elbow was also divided longitudinally into two halves, the division being aligned with that of the grid. Moisture was controlled by adding wet or dry pads of excelsior in the halves of the elbow. When the whole grid was heated, the highest numbers landed on the screen at temperatures about 4.5° C above the room temperature of 26—27° C (80—82° F) (R.H. 49%) and at an air speed of 13 cm/sec. Above 32° C (90° F) the numbers became less very steeply, and above 36° C (96° F), 2.5 degrees below blood temperature, no mosquitos ever remained on the screen for long. The effect of increasing the humidity to 65% when the whole grid was heated was to reduce slightly the numbers on the screen, but when the air through one half of the screen was moistened and the grid on that side heated to equalize the temperature on the two sides a preference for the moister side was manifest, at least at temperatures up to about 32° C (90° F) — this was influenced however by wind speed. A typical series of counts is given in Table VIII.

TABLE VIII

The numbers of A. aegypti *landing on each half of the upwind screen at various temperatures when one half of the stream was humidified. Illumination: 10 ft. candles.*

Air speed cm/sec.	Temp. °F. (both halves)	No. on Dry side	No. on Wet side	R.H.	Room Temp. °F.	R.H.	Cage Temp. °F.
0	77	0	0	61	77.5	67	77
7.5	85	6	11	—	—	—	—
13	82	23	30	—	—	—	—
40	81.5	29	31	—	—	—	—
40	90	6	5	—	—	—	—
13	103	0	0	—	—	—	—
7.5	100	3	0	—	—	—	—
0	98	1	2	47	80	51	81

The slight preferences for the wet side at the lower temperatures and for the dry side at the higher temperatures are not significant.

When half the grid was heated, over the whole range studied, 27° C to 38° C (82° F to 100° F), more mosquitos settled on the cooler half; a difference of 1° C was sufficient to cause a significant effect. When there was no air flow there were no differences, and when the experiment was repeated with the screen in a horizontal on top of the elbow to eliminate radiation, the results were essentially the same.

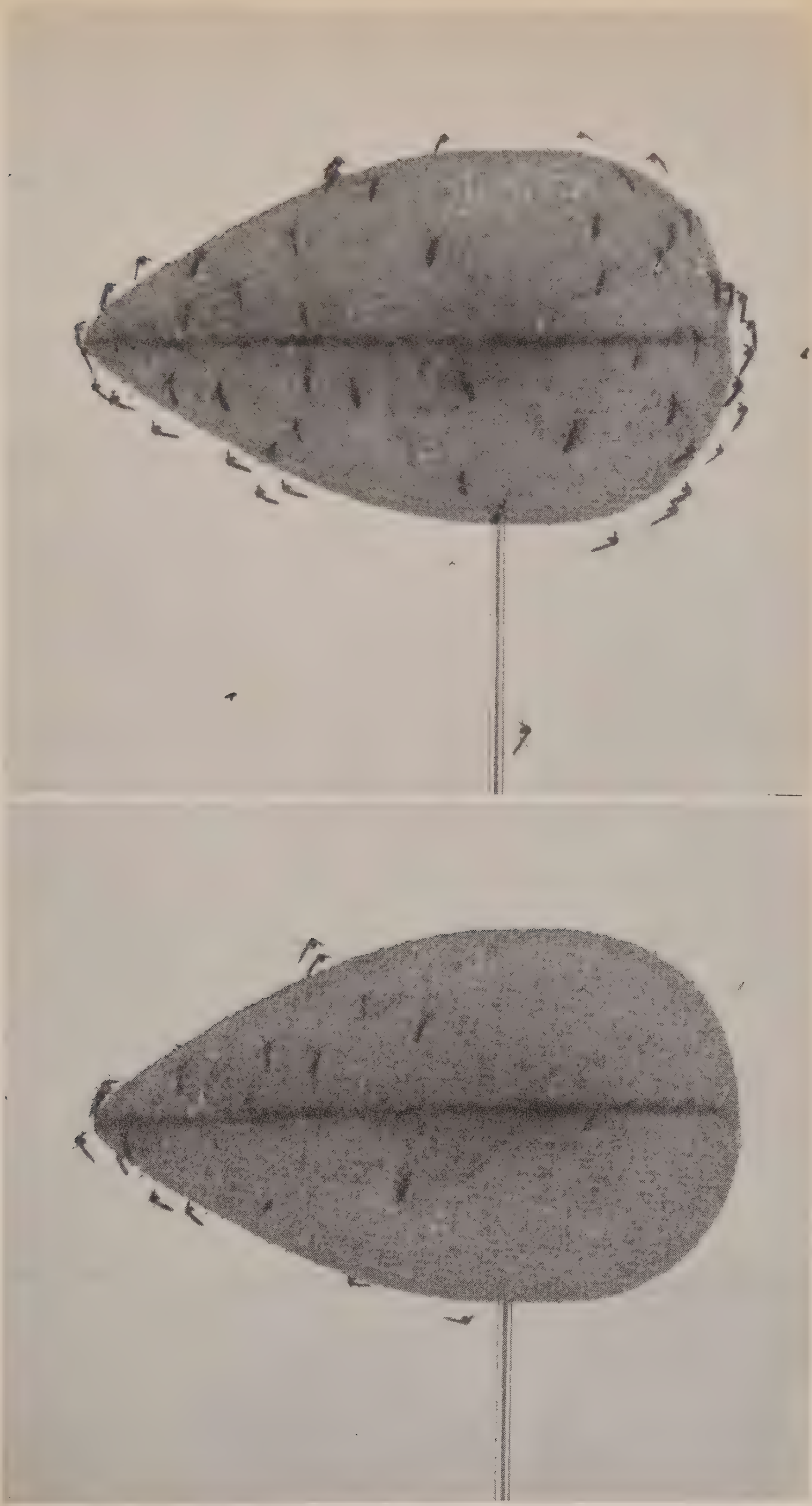

Fig. 2. The distribution of *A. aegypti* on a streamlined body in still air and in a wind of 13 cm/sec. Actual size.

Tests were also conducted with various proportions of carbon dioxide added to the air flowing through either half and through both halves of the inlet screen (cf. REEVES, 1953). No conclusive effects were manifest (WILLIS, 1947; WILLIS & ROTH, 1952).

By way of confirmation of the results obtained with the wind tunnel cage, the temperature and relative humidity in a second cage with no airflow were raised to 37.8° C (100° F) and 100% R.H. by placing a pan of water with a 400 watt immersion heater in it in the centre of the floor. An arm was exposed in the cage at intervals during the subsequent cooling down process as a test for biting response. The first apparently spontaneous flight was noted when the temperature had fallen to 32° C (90° F), and the first mosquitos landed on the arm at the same temperature. No biting was experienced until the temperature had dropped to 29.5° C (85° F).

Studies were conducted in the field using polyethylene bottless of three sizes. These were filled with water and could be heated electrically either from a 6 volt battery or through a transformer from the mains, by a coil of nichrome wire. Each was equipped with a dial thermometer and a number of sleeves of black, white, and red wool jersey were made to fit each size. Bottle dimensions are shown in Table IX and Fig. 3 shows the reflectance of the 3 fabrics from 3300 to 7000 Å.U. Typical figures for green vegetation taken from POPP & BROWN (1936) are included for comparison.

TABLE IX

Data on polyethylene test bottles

	Diam. cm	Height cm	Approx. watts to maintain at blood heat	Surface area used in experiment cm^2	Volume cm^3
Large	36	46	—	6100	46,800
Medium	15	25	65	1400	4,430
Small	10	20	12	680	1,590

Two medium bottles were used in a test of the effect of movement. These were covered with clean nylon stockings and suspended side by side from a tree in a wooded area by 7 m of nylon cord. In a series of counts of the numbers of wild *Aedes* sp. females sitting on a swinging and a stationary bottle as compared with counts when both were still the following figures were obtained:

Swinging	Check	Stationary	Check
73	67	50	65

This effect could only be demonstrated at a low amplitude (displacement about 1 m, maximum linear speed about 1 m/sec., the period was 5.5 sec.). When smell was added to the nylon stocking on the swinging bottle by holding this in the armpit while running 100 m, the swinging bottle then attracted more than twice as many as the stationary one.

In a second experiment, one bottle of each size heated to about 40° C (104° F) was placed at each corner of an equilateral triangle of side 1 metre on a table in a wooded area. Counts were made of the numbers of mosquitos sitting on each bottle with all possible combinations of size, position, and colour of covering sleeve. The results are summarized in Table X.

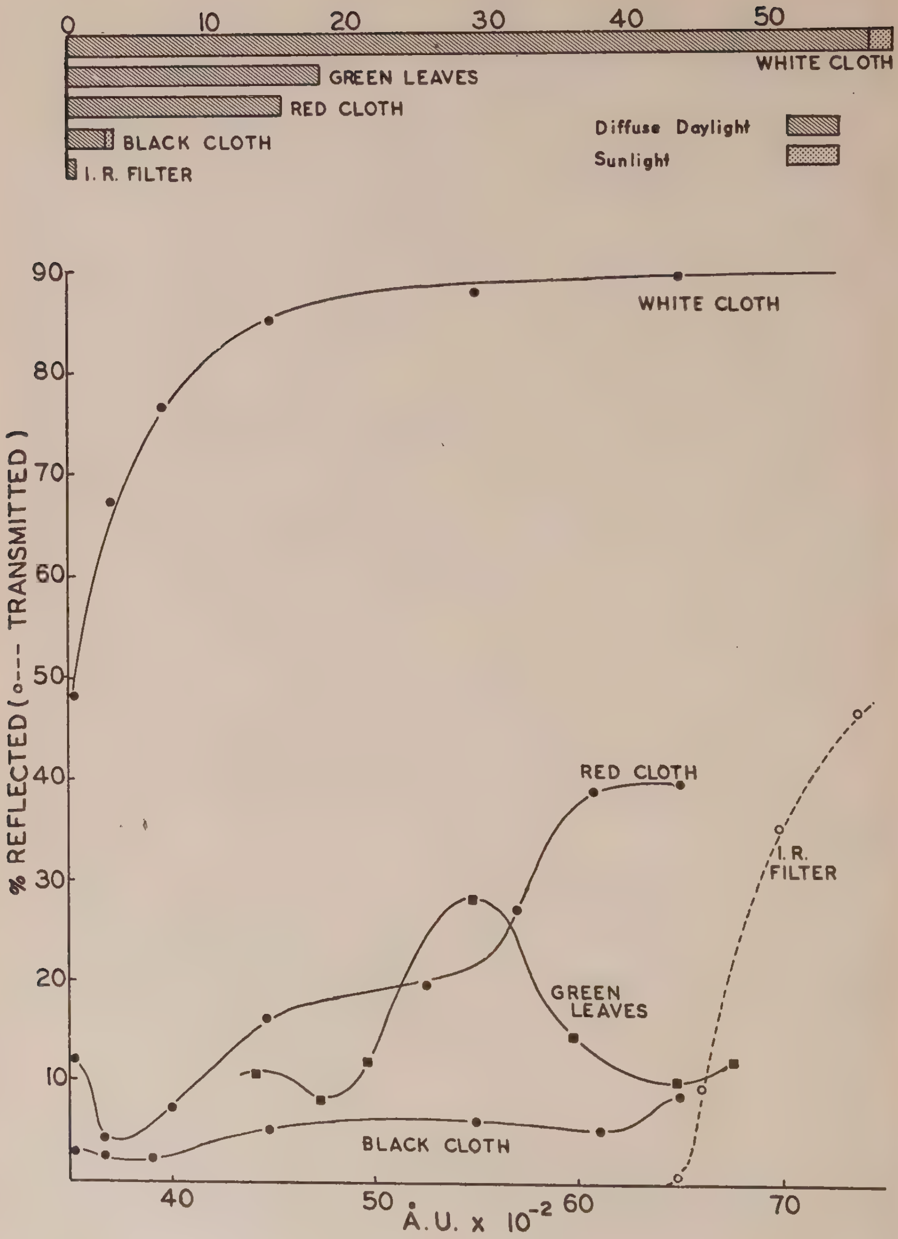

Fig. 3. The per cent reflectivity of coloured cloths (appendix 8) compared with green leaves and the transmission of Wratten No. 70 I.R. filter (appendix 9). Above, total reflectivity and transmission in the visual range; below, spectral distribution.

TABLE X

The numbers of Aedes *spp. females landing on warmed polyethylene bottles of various size, covered with various colours of wool jersey*

	White	Red	Black	Temp. Range	Total	Total per sq. cm
Mean temp.	107° F	106° F	105° F			
Large	24	70	26	109—104° F	120	0.0197
Medium	5	17	10	111—104° F	32	0.0228
Small	7	16	3	110—101° F	26	0.0383
Total	36	103	39			

The greater attraction of the red covering is highly significant ($P < 0.001$). The differences caused by size of bottle are not quite significant.

In a third experiment a small bottle with red jacket was placed in a cage of *A. aegypti* at ambient temperature and was slowly heated electrically to 48° C (126° F) and then allowed to cool to ambient while the numbers of mosquitos on the bottle were recorded at intervals. The results are shown in Table XI; it should be noted that the temperatures are those of the contained water, not surface temperatures.

TABLE XI

The numbers of A. aegypti *sitting on a small polyethylene bottle of water, covered with red wool jersey, at various temperatures*

Series 1, Dry

Temperature °F	70	82	89	97	116	126	110	100	95	76
Time	0800	0900	0930	0952	1101	1200	1231	1300	1320	1600
Number	61	72	76	78	45	25	41	65	66	56

Series 2, Wet (but dry by 103° reading)

Temperature ° F	74	75	82	92	95	108	116	103	98	91
Time	0800	0900	0930	0952	1101	1200	1231	1300	1320	1600
Number	28	54	32	37	38	29	52	74	58	50

In the dry series the numbers at the 4 temperatures nearest blood temperature are barely greater than at the lower temperatures (P about 0.4), but significantly exceed those at the higher temperatures ($P < 0.001$).

Field observations in another area showed that the peculiar attractiveness of red wool jersey extended also to *Anopheles earlei* Vargas.

APPENDIX 9

Optomotor and visual reactions. Kalmus (1958) has reported that *A. aegypti* shows responses to the rotation of the plane of polarisation of light.

Another interesting effect observed is that of assembled or swarming flight close underneath a weak light source placed on top of an otherwise darkened cage. This was shown most strongly immediately after the cage had been brightly lit (30 foot candles) and when a diffuse source was used (40 watt frosted 110 volt lamp run at 60 volts, close to a diffusing screen). The miniature swarms which formed below this source could be made to follow the source as it was moved about on top of the cage. When a 100 watt lamp run at 120 volts was used above a Wratten No. 70 red filter, no such reaction could be obtained although the total light transmitted was about 2½ times greater. The transmission of this filter is also shown in Fig. 3.

When black opaque objects were placed in various positions on the top of the cage with weak diffuse illumination some aggregation was seen, but attention seemed to be directed towards the margins of the objects.

APPENDIX 10

Searching on foot. Mosquitos settled on the upwind screen of the wind tunnel could be shown to orient towards and to move towards warm objects such as a finger or a heated test tube. The reverse reaction could also be shown to a tube full of iced water and to wet cotton.

To study these reactions further a means of controlling surface temperature was devised. A grid of aluminium foil strips was cemented to a card back with shellac tissue, and covered with red paper also cemented with shellac tissue. Flexible copper wire leads were connected to either end of the grid. Cards were 23 cm square; grids consisted of either 5 mm strips separated by 5 mm or 1 cm strips separated by 1 cm and covered one half only of each card. Electrical resistances of 1 ohm and 1.5 ohms were used, and 6 volt D.C. was applied with a variable resistance in series. The position of the grid could just be seen through the red paper. Temperature differences up to 12 Fahrenheit degrees were observed between the strips and the spaces. It was just possible for *A. aegypti* to land on the 1 cm grid with all four tarsi on a strip, or with all four tarsi on a space, and hence all at similar temperatures; with the 5 mm grid this was not possible. Cards were placed over the inlet to the wind tunnel cage after removing the screening; there was no wind. With the 1 cm grid most mosquitos landed on the cold strips and then oriented and moved onto the warm strips, on which, if they were vertical, they walked upward. Walking activity was much greater on the heated half of the card than on the unheated, and on the 5 mm grid on which there was always a temperature differential between tarsi than on the 1 cm.

If convection currents over the surface were reversed by a suction fan rotating slowly below (cf. appendix 5, p. 12) mosquitos still walked upwards.

When the grids were arranged horizontally mosquitos with their heads upwards would walk sideways along the strips of heated metal. Cards which had been held inside the shirt for 5 minutes to impart odour attracted more mosquitos. If these were wetted most mosquitos would land first on the heated half, but as this dried out they would gradually transfer to the cold, but still moist and smelly half. Mosquitos on the heated strips probed frequently.

APPENDIX 11

Tethered and enclosed mosquitos. The reactions of individual mosquitos were studied in small transparent boxes with various holes in them and by attaching a fine wire to the mesonotum and suspending the wire in such a way that they were free to fly and to rotate (Fig. 4).

In plastic (polystyrene) cages 2 cm × 2 cm × 15 cm, with a 6 mm diameter hole in one end covered with 28 mesh saran screening, *A. aegypti* and some specimens of field caught *A. fitchii* would immediately approach the hole and probe when the finger tip was placed about 3 mm away from it. The first part of this reaction could also be obtained if the finger was covered with dimethyl phthalate but the approach would cease at about 1 cm from the finger and was followed by confused activities.

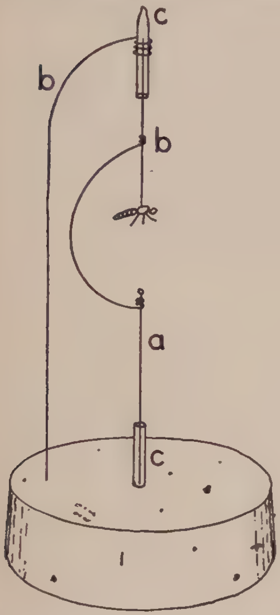

Fig. 4. The mount used for observations on individual insects in flight (appendix 11).
a. insect pin; b, fine aluminium wire; c. glass capillaries, sealed at one end.

When a finger is brought near a suspended flying mosquito it turns towards it and grapples for it with the legs. If the finger is covered with dimethyl phthalate it turns away, but not usually until it has made contact with the repellent. If repellent is presented on a glass rod, turning away is observed at a distance of about 1 cm. When a neutral material such as filter paper is brought up under the tarsi, the mosquito will grapple for this and settle, and cease flying when contact is made. If the paper carries diethyl toluamide, indalone, dimethyl phthalate, or ethyl hexanediol, no reaction is observed until contact is made, the legs are then lifted and removed from the paper as long as this is possible. Flight continues, or ceases only momentarily. If a drop of repellent is added to clean filter paper on which a mosquito is standing, so as to reach its tarsi one by one by capillarity, flight always starts when the repellent reaches the last tarsus, and may start sooner than this.

If a droplet of repellent is applied to the tip of the proboscis an intense wriggling activity of this organ ensues.

APPENDIX 12

Other experiments with repellent chemicals. In a series of field experiments target areas were drawn out on the backs of subjects who wore shirts with the backs cut out. A central circle of 4 cm radius (50 sq. cm) was drawn in a hard clear nail varnish, using ink compasses. Concentric with this a series of circles were drawn with a black ball-point pen, so that each successive ring had an area of 50 cm². So long as the subject was lying face downwards, the nail varnish circle prevented repellent painted inside it from spreading. Under normal conditions one observer could keep check on four subjects, marking the positions of bites, once these were established, with a camel hair brush dipped in methyl violet. Tests were conducted in a clearing in a wooded area where the temperature ranged from 14 to 21° C (57 to 69° F), the relative humidity from 55 to 89%, and the wind speed was never more than Beaufort 2. The total numbers of bites recorded in each of the equal areas in relation to the distance from the repellent treated area is shown in Fig. 5. It was not possible to demonstrate any influence of wind direction or speed on the distribution of bites in relation to repellent.

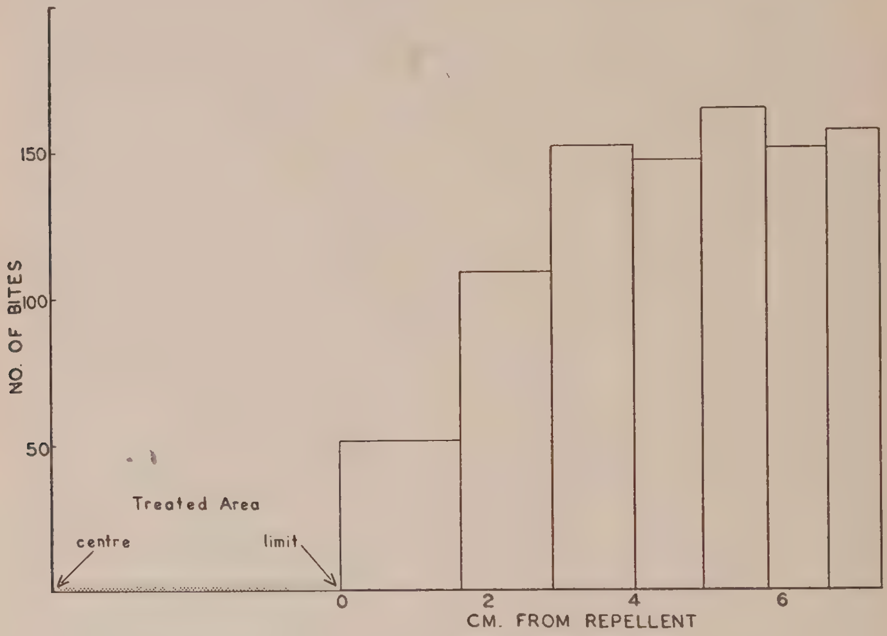

Fig. 5. The distribution of bites by wild *Aedes* spp. in relation to distance from a small repellent treated area.

A guinea pig confined in a small plastic cage was placed in the wind tunnel cage on two wooden pillars 15 cm high so as to bring it within the wind column. A band of ethyl hexanediol about 1 cm wide painted around each pillar near the top was almost completely effective in protecting the guinea-pig.

Tests were run with dimethyl phthalate in a T-tube apparatus (HOCKING & LINDSAY, 1958); these gave an olfactory response figure of —0.35. When half the air flow was over the socks used in appendix 5 and half over dimethyl phthalate the figure obtained was +0.38 and when the guinea pig replaced the socks it was +0.33.

When wood shavings soaked in dimethyl phthalate were used to impart odour to the wind in the wind tunnel cage, very few mosquitos landed on either the upwind or the downwind screen under any conditions of light or wind speed. Very few mosquitos settled on a guinea pig when it was placed in such an odorous air current. Tests were conducted with a second screen of 2 mm mesh saran covering half of the upwind screen, about 2.5 cm from it, which could be painted with dimethyl phthalate. Tests were repeated with the second screen, both treated and untreated, in each of four possible positions. The results are shown in Table XII.

TABLE XII

The effect of odour of dimethyl phthalate and of wind speed on the numbers of A. aegypti *landing on a screen. Each figure is the sum of four readings on a half-screen*

Wind speed cm/sec.	Untreated double screen	Control single screen	Dimethyl phthalate treated double screen	Control single screen	Totals
0	20	19	9	47	95
13	188	267	35	201	691
48	176	198	58	139	571
Totals	384	484	102	387	

The differences between columns 3 and 5 can be attributed to differences in the previous treatment of the insects. The effects of the repellent are highly significant at all 3 wind speeds ($P < 0.001$).

When two petri dishes covered with gauze, one of which contained dimethyl phthalate, were placed on a hot plate in a cage of *A. aegypti*, significantly fewer mosquitos landed on the gauze over the repellent.

Four series of tests were done with dimethyl phthalate, ethyl hexanediol, indalone, and diethyl toluamide in conjunction with a hand in a cage without wind. In the first a 1 cm diameter circle was drawn on the back of the hand with nail varnish, a rubber glove with a 6 cm diameter hole in the back was put on, and one drop of repellent was applied within the nail varnish circle. The hand was then rested on the floor of the cage and mosquito behaviour observed. Bites were obtained almost up to a mosquito half-width from the circle except where depressions between veins and sinews adjoined this. At such places there was sometimes biting 1 to 2 cm of the circle. This test was not satisfactory with ethyl hexanediol which dissolved the nail varnish and crept over the skin. In the second tests a 10 cm length of 3 mm O.D. glass tubing was clamped in a vertical position so that the lower end was about 1 cm above the middle of the 6 cm bare circle on the back of a gloved hand. A few drops of the repellent were placed in the lower end of the tube. In this way, a circle of skin about 15 mm diameter was kept free of bites, but the margin of the circle was modified by the contours of the hand. This circle was less well marked with diethyl toluamide than with the other materials. In the third tests liquid repellents were painted on the backs of blood-feeding mosquitos with a fine camel-hair brush. Results were variable, but with all materials except diethyl toluamide, proboscides were withdrawn and mosquitos took off when an area a little greater than the mesonotum had been covered. This seemed to be independent of the position of the application. With diethyl toluamide some mosquitos continued feeding even when their wings were stuck together with the liquid. In the fourth tests rubber gloves with 12 mm diameter holes in the back were worn and the behaviour of *A. aegypti* in the cage was observed when the exposed portion of skin was untreated and treated with each of the four repellent chemicals investigated and the hand was held in the cage. No difference between these were observed. Typical results observed with ethyl hexanediol are given in Table XIII.

TABLE XIII

Responses of A. aegypti *to a 12 mm circle of exposed skin, with and without repellent*

	Untreated	Ethyl hexanediol	Untreated	Ethyl hexanediol
No. settled on circle of hand	15	0	15	0
No. searching on the wing	4—6	2—5	3—4	5—6

The repellent action is strong and highly significant as far as settling is concerned ($P < 0.001$), but no effect is apparent in respect of searching.

When a 3 mm band of the rubber glove around the rim of the hole was painted with diethyl toluamide and the skin was untreated many mosquitos landed directly on the skin, probed, and bit. Those landing with any of the tarsi touching the repellent usually took off. Those landing outside the ring and searching by walking never crossed the ring.

Acknowledgements

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ZUSAMMENFASSUNG

VERHALTEN VON AEDES *MÜCKEN IN BEZUG AUF BLUTSAUGEN UND REPELLENTS*

Weibchen von *Aedes aegypti* sowie einiger Kanadischer *Aedes*-Arten wurden im Freien und im Laboratorium beobachtet. Ihr Verhalten in bezug auf verschiedene Opfer und auf abstossende Substanzen wird in die folgenden acht Phasen unterteilt: Ruhe, Flug, spontanes Suchen im Flug, Landung auf dem Wirt, Suchen zu Fuss, Untersuchung mit dem Rüssel und Stich. Die Hierarchie dieser Verhaltensweisen sowie ihre gewöhnliche Aufeinanderfolge werden diskutiert. Die oben beschriebene Reihenfolge scheint die natürlichste und ist vermutlich am häufigsten. Aber je nach den Umständen kommen auch andere Teilfolgen vor und insbesonders können einzelne Phasen ausbleiben. Unter den für die einzelnen Verhaltensweisen — oder besser gesagt Verhaltensübergänge — massgebenden Faktoren wurden Oberflächentemperatur, Konvektions- und Strahlungswärme, Licht, Farbe, Feuchtigkeit, Geruch, Geschmack, Schwerkraft, Schall und Bewegung geprüft und in bezug auf ihre Effekte untersucht.

Die Bedeutung dieser Ergebnisse und der gesamten Problemstellung für die Ausarbeitung verbesserter Mücken-abstossender Chemikalien wird diskutiert. Offensichtlich ist das Problem des Schutzes gegen Insektenstiche nicht einfach eine Frage der Chemie und kann nicht durch das übliche Herumprobieren mit gekäfigten Mücken gelöst werden. Prinzipiell können die Verhaltensketten sowohl der weiblichen Mücke als auch des Menschen, die gemeinsam zum Stiche führen, an mehreren Stellen und in mannigfaltiger Weise unterbrochen werden, und zwar sowohl im Freien als auch in Häusern oder Zelten. Unter diesen Möglichkeiten ist der Gebrauch abstossender Substanzen nur eine, wenn auch wichtige Hilfe.

Aber selbst auf diesem beschränkten Gebiet müssen mehr Variable berücksichtigt werden, als dies gewöhnlich geschieht. Die Entscheidung, ob eine Mücke sticht oder nicht, kann als Ergebnis eines Wettstreits antagonistischer Faktoren verstanden werden. In diesem Zusammenhang ist es wichtig, dass die gleichen Substanzen, z.B. in Abhängigkeit von der Konzentration, abstossend oder anziehend wirken können, und dass sie die verschiedenen Verhaltensphasen nicht immer gleichsinnig beeinflussen, sodass etwa eine Substanz, die das Stechen verhindert, nicht auch das Niederlassen unterbinden muss. Endlich muss man in Betracht ziehen, dass sowohl der Stechtrieb der Mücken als auch die Anziehung durch das Opfer mannigfaltigen und komplizierten Schwankungen unterliegen, von denen nur einige bekannt sind.

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ACCLIMATIZATION TO COLD IN INSECTS[1]

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Evidence is given that insects acclimatized to colder temperatures are more resistant to immobilization by cold. There is a temperature below which further acclimatization is not achieved. Data are presented for the rate of gain and loss of cold tolerance. This is further evidence of acclimatization. The rate of recovery after immobilization by cold is related to the acclimatization temperatures. The rate of survival at low temperatures above freezing is dependent upon the acclimatization temperature.

BULLOCK (1955) pointed out the lack of experimental evidence on temperature acclimatization in insects. Much of the evidence so far deals with seasonal effects. BODENHEIMER & KLEIN (1930) showed that the activities of ants in Palestine varied according to the month. AGRELL (1941) demonstrated that Collembola were most resistant to cold in winter and LEES (1948) determined that sheep tick nymphs collected in winter became immobilized at a lower temperature than those collected in summer.

It was MELLANBY (1939, 1958) who showed for a variety of insects that their chill-coma temperature depended upon their previous temperature history. The same worker (1940) extended this laboratory work to a number of arctic species in the field. This work suggested that insects acclimatize to temperature.

The work of MELLANBY (*loc. cit.*) has been extended in some detail to a laboratory study on acclimatization to cold in *Blattella germanica* L. Preliminary communications on this problem were given by COLHOUN (1954 and 1956).

METHODS AND MATERIALS

A culture of *Blattella germanica* was maintained in a large metal tank containing small earthenware pots. They were provided with food and water but no attempt was made to separate the stock into age groups. An electric fence prevented the escape of roaches into the rearing room. The air temperature of the tank was 25 ± 2° C; humidity was not controlled but averaged 70 per cent. Male roaches were used for experiments and these were taken at random from the stock culture.

The accuracy of the experimental work depended upon a sensitive and constant temperature apparatus. Accordingly, an apparatus was constructed which had the following essential features. A series of glass-sided water baths were constructed. They contained both heating and cooling coils. A cooling unit was designed from a Kelvinator refrigeration unit, which cooled a reservoir of glycerine and water. This mixture, termed the coolant, was pumped through the cooling coils arranged in series so that the coldest bath was next to the refrigerator unit. Temperatures were obtained by thermostatic control to 0.05° C. Mercury toluene thermostats were used and the degree of temperature control depended largely upon an electronic unit which operated mercury relays controlling the heaters. Any desired temperature was easily obtained with this unit and accurately held for any given period.

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[1]) Contribution No. 113.

Roaches were acclimatized to temperature in the following manner. They were removed from the stock culture and lightly anaesthetized with carbon dioxide; then placed in groups of five within 200 cc bottles, the bottoms of which were covered with filter paper. The bottles were attached to a wooden rack and as soon as the roaches had recovered from the anaesthetic, the rack was placed over a water bath so that the bottles were fully submerged. The bottles were connected in series by the use of rubber tubing; air of the same temperature as the bath was slowly circulated through the bottles. At all acclimatization temperatures the saturation deficiency of the circulating air was kept at 7 mm Hg. This was obtained by placing large jars, containing sulphuric acid solutions, (WILSON, 1921, SOLOMON, 1951) in each water bath and by passing air through these and then into the bottles containing roaches. At very low temperatures condensation was prevented by circulating dry air through the bottles for 15 minutes.

The effect of acclimatization at temperatures of 35° C, 25° C, 15° C and 10° C was tested by transferring the rack and attached bottles containing roaches to a selected low temperature. The activity of the roaches was observed at given intervals after the temperature change. A roach was considered to be in chill-coma if it was incapable of standing up or able to crawl. For the purpose of this work all others were considered to be active. The response of roaches was observed by tapping the bottle with a glass rod.

Probit analysis, according to the method of WILCOXON & LITCHFIELD (1949), was used to determine the ED_{50} of activity at low temperatures.

RESULTS

Relation between acclimatization temperatures and chill-coma temperatures

Roaches were held at their acclimatization temperatures for extended periods to determine mortality. It was found that they died more quickly at 35° C than at either 25° C, 15° C or 10° C. At 168 hours all survived at the latter temperatures whereas at 35° C, 35 per cent had died at 72 hours; at 168 hours all were dead at 35° C. These results showed that prolonged acclimatization at 35° C would injure *Blattella.* After transfer from the stock culture at 25° C, it was determined that 24 hours was sufficient to acclimatize roaches at 35° C. The injury effect of long exposure to 30° C was therefore avoided.

It was necessary to determine the period of exposure to low temperatures before determining activity. This was accomplished by taking samples of roaches from each acclimatization temperature and by placing them at predetermined low temperatures. Their activity was recorded for 48 hours at the low temperatures. The results showed that some roaches eventually recovered from chill-coma induced by the effect of the change in temperature from the acclimatization temperatures to the cold temperatures. An example of this is given in Fig. 1 where roaches acclimatized at 25° C were transferred to 7° C. After 15 minutes at 7° C a number of the roaches were observed to be prostrate, but three hours

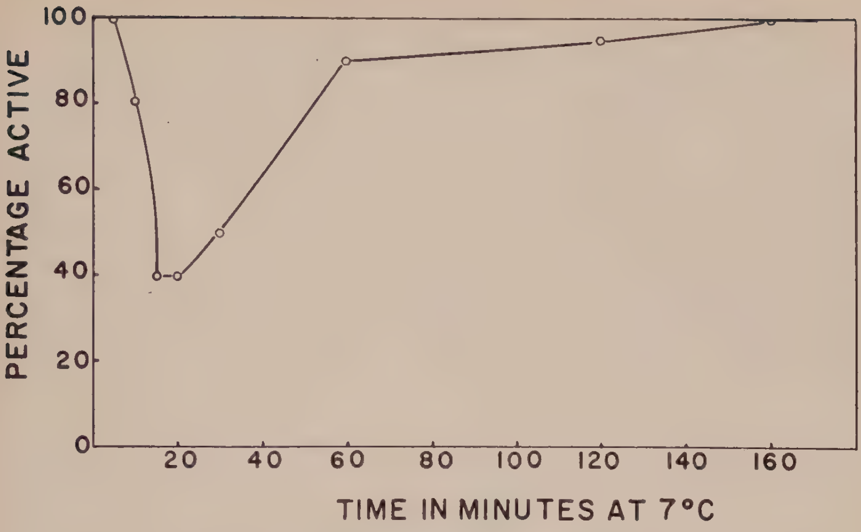

Fig. 1. The relation of activity and time of exposure at 7° C for roaches, acclimatization at 25° C.

later they had recovered. From these initial studies it was determined that 24 hours was sufficient to record activity at low temperatures.

Mellanby (1939) determined the chill-coma temperature after an exposure of only 15 minutes; my results show that he would have obtained more precise data after a longer exposure, though Mellanby informs me (personal communication) that the difference between the chill-coma temperature as determined by a 15 minute or a 24 hour exposure is in many insects only in the region of 1° C.

The effect of acclimatization upon activity at low temperatures is illustrated in Fig. 2. It is evident that roaches previously held at 15° C were more resistant to cold than those acclimatized to 25° C and 35° C. The temperature at which 50 per cent of the roaches were able to remain active (ED_{50}) was determined from the regression curves in Fig. 2. These temperatures were plotted, and as shown in Fig. 3, the relation was linear. Mellanby (1939) based his observations on the chill-coma temperature rather than upon the ED_{50} level used in the present work. The chill-coma temperature was defined as the temperature at which the insect was immobilized by cold. For *Blattella*, the observed chill-coma temperatures are given in Table I. When plotted against the acclimatization temperatures (Fig. 3), the relation between these temperatures and the chill-coma temperatures is nearly linear, and in this respect similar to the results obtained with the ED_{50} level of activity.

The results obtained from chill-coma temperature showed that roaches kept at 10° C had the same chill-coma temperature as those acclimatized to 15° C (Fig. 3). Therefore, there is a temperature below which further acclimatization does not take place. For *Blattella* this appears to be 15° C. An analysis of the results in Table I also showed that the degree of acquired cold resistance for the temperature range of 15° C to 35° C was 2.6° C. For each 1° C of acclimatization

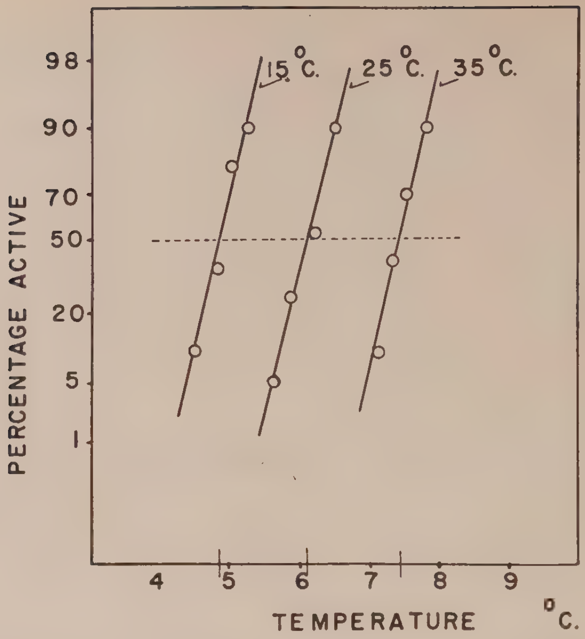

Fig. 2. Probit analysis of activity and cold temperature for roaches acclimatized at various temperatures.

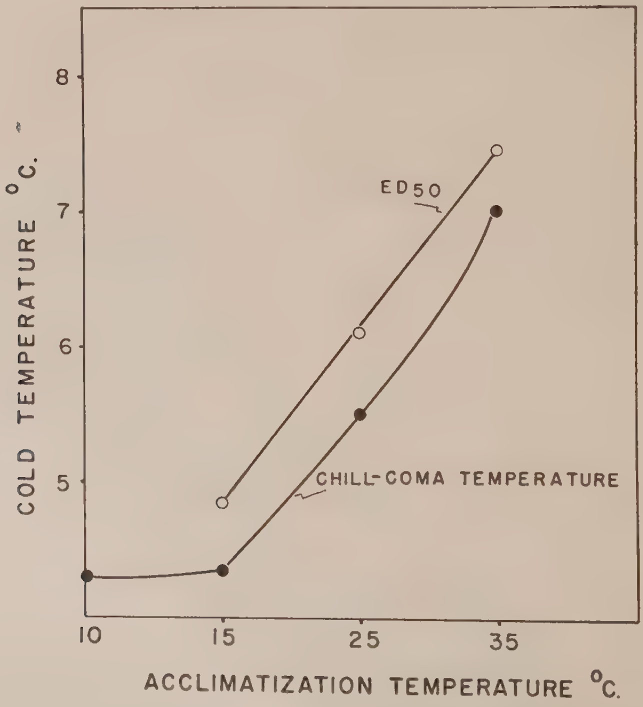

Fig. 3. The relation between ED50 of activity, chill-coma temperatures and acclimatization temperatures.

TABLE I

The chill-coma temperatures for males of B. germanica *acclimatized at various temperatures*

Acclimatization temperature	Chill-coma temperature
35° C	7.0° C
25° C	5.5° C.
15° C	4.4° C
10° C	4.3° C

over the range of 15° C to 35° C, 0.13° C of cold acclimatization was acquired. These results are in sharp contrast to those of FRY, BRETT & CLAWSON (1942) who determined that goldfish acquired 2° C of cold acclimatization for each 3° C of acclimatization temperature.

The rate of acclimatization

The possibility existed that the data illustrated in Fig. 2 might be due only to the shock effect of transferring roaches from their acclimatization temperatures to cold temperatures. Experiments were then carried out to determine the rate at which roaches gained or lost their resistance to cold. The method followed was that of BRETT (1946) who used the terms, gain of cold tolerance, and loss of cold tolerance. Temperatures for testing the rate of gain and loss of cold tolerance are given in Table IIa, b. For example, it was shown that roaches acclimatized to 15° C were able to remain active at 5.4° C, whereas those acclimatized to 25° C were in chill-coma at this temperature. Therefore, roaches acclimatized to 15° C and then transferred to 25° C were said to have lost their cold resistance when they were unable to remain active at 5.4° C. In practice, roaches were transferred from the old acclimatization temperatures to the new acclimatization temperatures for chosen periods and then their activity was tested at low temperatures. These results are given in Figs. 4 and 5. A comparison of these data shows that the gain of cold tolerance was achieved more quickly than it was lost in the temperature

TABLE II

Temperatures for determining the rate of loss and gain of cold tolerance (a) the loss of cold tolerance (b) the gain of cold tolerance

(a)

Old acclimatization temperature	New acclimatization temperature	Temperature of zero activity at 24 hours
15° C	25° C	5.4° C
25° C	35° C	7.0° C
15° C	35° C	7.0° C

(b)

Old acclimatization temperature	New acclimatization temperature	Temperature of 100 per cent activity at 24 hours
35° C	25° C	7.0° C
25° C	15° C	5.5° C
15° C	15° C	5.5° C

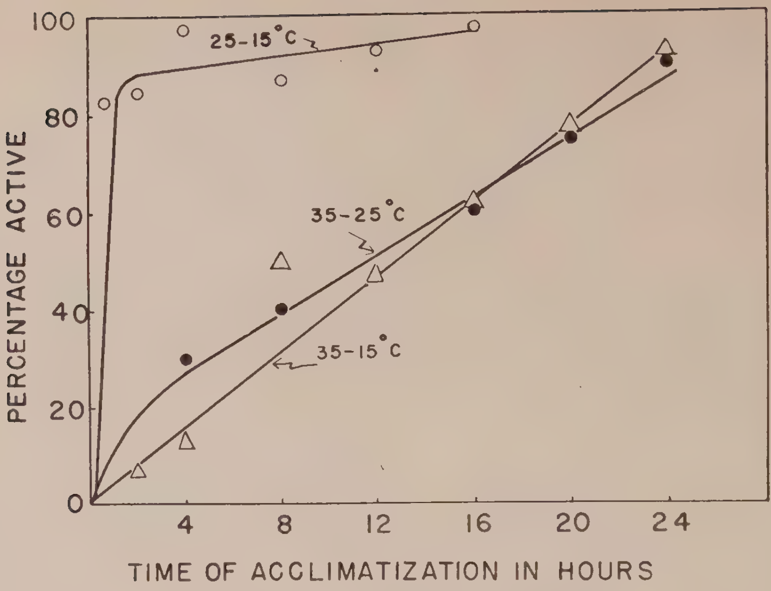

Fig. 4. The rate of gain of cold tolerance.

changes of 25° C to 15° C and 35° C to 15° C, but lost more quickly for the temperature change of 25° C to 35° C. The significance of these results points to the fact that after a change from one acclimatization temperature to another a time factor is involved before acclimatization is complete. This is characteristic of adaption phenomena in general. It rules out the possibility that the results given in Fig. 3 were due only to the rapidity and degree of temperature change. There are no other data for the rate of cold acclimatization in insects. It is possible that cold is the more important temperature limiting factor for *Blattella*, which

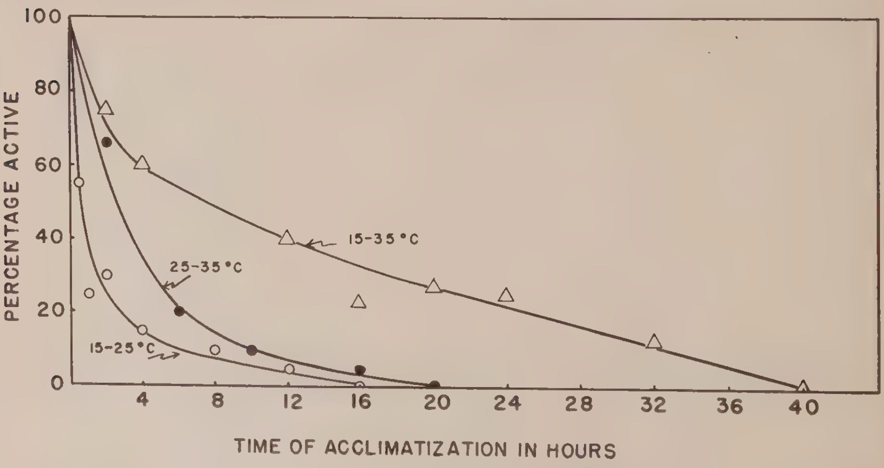

Fig. 5. The rate of loss of cold tolerance.

prefers a warm environment (Gunn, 1935). Therefore a quick gain of cold tolerance may have survival value.

Recovery from chill-coma

Mellanby (1939) briefly indicated that there was a relation between the acclimatization temperature, the chill-coma temperature and the temperature at which the insects were allowed to recover from chill-coma. The results given in Table III illustrate this hypothesis. It was further found that the rate of recovery from chill-coma was much slower if roaches were left at chill-coma temperatures for 5 hours. These results are given in Table IV. It is also apparent that the rate of recovery was slower for roaches kept at the colder acclimatization temperatures. These results differ from those of Mellanby (1939) who showed that *Blatta orientalis* recovered immediately regardless of whether it was immobilized for either 24 or 72 hours if cooled to just below the chill-coma temperature; he found that recovery from lower temperatures was much slower. It was also found here that the recovery temperature determined the rate of recovery from chill-coma. This is shown in Table V where roaches acclimatized at 35° C recovered much more slowly from their chill-coma temperature when placed at 15° C. Conversely roaches acclimatized at 15° C recovered more quickly at 35° C. A comparison between Tables IV and V clearly illustrates this point. It is significant that roaches acclimatized at 35° C recovered much more slowly at 15° C than those acclimatized at 15° C and allowed to recover at 15° C. The results from these experiments

TABLE III

The rate of recovery for males of B. germanica *after a 15-minute exposure to chill-coma temperatures*

Acclimatization temperature	Chill-coma temperature	Recovery temperature	Mean recovery time in mins.	Standard error of the mean
35° C	6.9° C	35° C	2.28	± 0.09
25° C	5.4° C	25° C	2.70	± 0.12
15° C	4.3° C	15° C	3.30	± 0.16

TABLE IV

The rate of recovery for males of B. germanica *after a five-hour exposure to chill-coma temperatures*

Acclimatization temperature	Chill-coma temperature	Recovery temperature	Mean recovery time in mins.	Standard error of the mean
35° C	6.9° C	35° C	6.38	± 0.51
25° C	5.4° C	25° C	9.05	± 0.32
15° C	4.3° C	15° C	22.13	± 1.27

TABLE V

The rate of recovery for males of B. germanica *at temperatures other than their acclimatization temperatures after a five-hour exposure to chill-coma temperatures*

Acclimatization temperature	Chill-coma temperature	Recovery temperature	Mean recovery time in mins.	Standard error of the mean
35° C	6.9° C	15° C	45.63	± 3.45
15° C	4.3° C	35° C	4.63	± 0.34

show that the ability of *Blattella* to recover from chill-coma is a function of their response to the recovery temperature. This response appears to be related to changes induced within the insect at the acclimatization temperatures, therefore providing further evidence that *Blattella* acclimatize to temperatures within their biokinetic temperature range.

Survival at low temperatures

Evidence obtained from the rate of recovery from chill-coma showed that prolonged exposure to cold might result in a difference in the rate of survival of roaches acclimatized at various temperatures. Roaches were acclimatized at 35° C, and 25° C and 15° C and then transferred to 7° C. The rate of survival was tested by removing samples of roaches and determining whether they recovered at 25° C. The results given in Fig. 6 show that roaches acclimatized to 15° C were more resistant to cold than those held at either 35° C or 25° C.

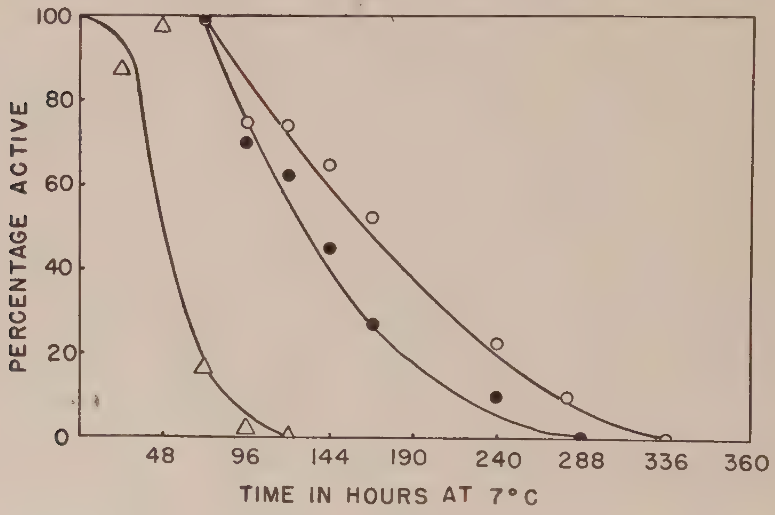

Fig. 6. Survival of roaches at 7° C.
○ acclimatized at 15° C
● acclimatized at 25° C
△ acclimatized at 35° C

However, the survival factor had limited value, for eventually all the roaches died. In principle the results show that acclimatization, which lowered the chill-coma temperature, also varied the cold-death point. The latter conclusion is of importance as, unless the temperature history of an insect has been taken into consideration, the data in literature on the cold-death point of insects are meaningless.

In the course of the experimental work little evidence was obtained upon the mechanism of cold acclimatization. Two observations gave indication that the nervous or muscular systems might be involved. It was noted that the onset of chill-coma brought about symptoms of convulsions which were particularly evident in roaches acclimatized to 35° C. The second observation of a possible nervous malfunction was obtained when roaches, held at low temperatures for a prolonged period, were allowed to recover at 25° C. A number of these roaches showed locomotory incoordination and pronounced tremors of limbs and other appendages. This result is given in Table VI. The roaches did not recover from the injury.

TABLE VI

The effect of cold upon males of B. germanica *acclimatized at 35° C, placed at 7° C, and allowed to recover at 25° C*

Exposure period in hours	Numbers of insects		Injury		
		Normal	Walking tremors	Prostrate tremors	Dead
5	40	40	0	0	0
24	40	35	0	0	5
48	40	21	0	19	1
72	40	6	0	1	33
96	40	0	1	0	39
120	40	0	0	0	40

DISCUSSION

In principle the present results confirm the work of MELLANBY (1939 and 1940), although it is evident that the short exposure periods to cold, used by this worker, are inadequate for the precise determination of chill-coma temperatures. The results show that for the acclimatization temperature range of 10° C to 35° C, the chill-coma temperature for *Blattella* is altered by 2.6° C. It is questionable that this slight modification in response to cold is of value as a survival factor to *Blattella.* On the contrary, FRY, BRETT & CLAWSON (1942) have shown that acclimatization significantly altered the ability of goldfish to survive at low temperatures, while a number of workers have shown that acclimatization of fish to temperature appears to have an important bearing upon survival in nature (BRETT, 1956).

It is well known that insects can be divided into categories depending upon their response to cold (WIGGLESWORTH, 1953). These are: those that die at temperatures above the freezing point of water, an example of which is *Blattella*; those insects that die when their tissues become frozen; and lastly a relatively small number of insects which seem able to withstand freezing. Insects placed in these arbitrary categories appear to live in different environmental conditions. *Blattella* belongs to "the warm loving" group of insects and therefore the species is only found under conditions of temperature and humidity suitable to survival. It would be surprising, then, that the lethal cold-death point for *Blattella* could be altered to any great degree by a period of acclimatization or conditioning at a fixed environmental temperature. The results obtained in the present experimental study amply confirm this.

With *Blattella* the experimental evidence appears to show that exposure to a constant temperature for a given period results in some intrinsic change in either the physiological or biochemical make-up of the insect. This indeed might be caused only by a steady state environmental condition, for in nature insects are rarely exposed to constant conditions. Most insects are able to avoid extremes of temperature. It would also appear that the most suitable temperature range for

Blattella is between 15° to 35° C, for this is the range within which it was possible to alter the response to cold.

There is very little evidence for the mechanism of alteration of the chill-coma point. Whatever changes do occur, they must be brought about at the acclimatization temperatures. SAYLE (1928) and MARZUSCH (1952) showed that adjustments in oxygen consumption were obtained when insects were acclimatized at various temperatures. Similar evidence was found by DEHNEL & SEGAL (1956) for adults and nymphs of *Periplaneta.* The adults acclimatized less than the nymphs. With temperature acclimatizations MARZUSCH (*loc. cit.*) claimed to have found changes in the rate of activity of the enzymes, succinodehydrogenase, catalase and glycero-phosphatase. MUNSON (1953) determined changes in the lipid content of *Periplaneta* although he was unable to show acclimatization to heat. An alteration in the amounts of a hormone of the central nervous system of *Periplaneta* was found by COLHOUN (1958), who showed that when males were kept at 35° C the level of acetylcholine increased over that found at 25° C. The level of acetylcholine was reduced when roaches were placed at a lower temperature.

The above information is evidence that certain changes occur within insects kept at different temperatures. The importance of these in an evaluation of the alteration of either the chill-coma temperature or cold-death point is unknown. Indeed it is questionable that the mechanism for the enhanced response to cold of only 2.6° C in *Blattella* could be easily determined.

Grateful appreciation is given to Professor V. B. Wigglesworth who suggested this problem and who provided encouragement during its progress. Thanks are accorded to Dr. J. W. L. Beament for technical advice and to the technical staff of the Entomology Department, Department of Zoology, The University of Cambridge. Financial assistance was provided by the Ontario Research Foundation, by the Department of Agriculture, Division of Entomology, and by the Department of Veterans' Affairs, Government of Canada.

ZUSAMMENFASSUNG

KÄLTE-AKKLIMATISATION BEI INSEKTEN

1. Die experimentellen Daten zeigen, daß *Blattella* nach einer Akklimatisierungs-Temperatur von 15° C resistenter gegen Kälte ist als nach einer solchen von 25° C oder 35° C.
2. Nach einer Akklimatisierungs-Temperatur von 10° C tritt Kältestarre bei der gleichen Temperatur ein wie nach einer Akklimatisierungs-Temperatur von 15° C. Dies zeigt, daß nach Unterschreiten einer Minimaltemperatur keine weitere Akklimatisierung stattfindet.
3. Die Geschwindigkeit, mit der eine Akklimatisation bei 15° C, 25° C und 35° C erfolgt, zeigt, daß die Depression der Temperatur für das Einsetzen der Kältestarre nicht durch einen Schockeffekt hervorgerufen wird.
4. Die Geschwindigkeit, mit welcher eine Erholung aus der Kältestarre stattfindet, weist darauf hin, daß *Blattella* sich an verschiedene Temperaturen akklimatisieren kann.
5. Die Reaktivierung von *Blattella* aus der Kältestarre wird bei niedrigen Temperaturen über dem Gefrierpunkt durch die Akklimatisierung beeinflußt. Nach einer Akklimatisierungs-Temperatur von 15° C überleben mehr Schaben als nach einer solchen von 25° C oder 35° C. Alle Schaben, die an eine Temperatur von 15° C akklimatisiert waren, starben schließlich bei 7° C, obwohl sie gegen Kälteeinwirkung resistenter waren als Schaben, die an eine Temperatur von 25° C oder 35° C akklimatisiert waren.

6. Wie in der Diskussion der Ergebnisse ausgeführt wird, ist es unwahrscheinlich, daß ein Senken der für das Eintreten der Kältestarre notwendigen Temperatur um 2.6° C für freilebende *Blattella* bedeutungsvoll ist.

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HOST PLANT PREFERENCE OF THE SOYBEAN POD BORER, *GRAPHOLITHA GLICINIVORELLA* MATSUMURA (LEP., EUCOSMIDAE)

1. Oviposition site

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The host plant preference of the soybean pod borer, an important factor in the resistance of the soybean plant, was studied in the field, using four varieties, two hairy and two glabrous. It was found that there was a considerable difference in the number of eggs laid on the hairy and glabrous varieties, and also in the place where eggs were laid. These differences were observed when the hairy varieties were planted alternately with the glabrous ones. However, the difference in the number of eggs laid was not found when all pods of the soybean plants were removed. This treatment of the hairy varieties caused a great decrease in the number of eggs laid, while on the glabrous varieties this was not the case. These observations suggest that two factors operate (a) varietal preference (b) site preference within plants, e.g. the hairy pod is the most attractive oviposition site.

PAINTER (1951) has stated that insect resistance in crop plants is due to three factors: preference, antibiosis and tolerance. This was confirmed in a previous paper (NISHIJIMA & KUROSAWA, 1953) dealing with the resistance of soybean varieties to the soybean pod borer, *Grapholitha glicinivorella* Matsumura, which is one of the most important pests of soybean in Japan. The experiment on the relative importance of these factors was conducted by the method of inoculation (NISHIJIMA, 1954). It demonstrated that the principal factor conferring resistance in soybeans was related to host plant preference as it concerned oviposition. Thus, an investigation was undertaken in order to investigate the problem.

The subject of host preference has occupied the special attention of many workers, particularly in relation to insect nutrition (DETHIER, 1953, 1954; FRAENKEL, 1953, 1958; HOUSE, 1958; KENNEDY & BOOTH, 1951; KENNEDY, 1953, 1958; LIPKE & FRAENKEL, 1956; PAINTER, 1953; THOMPSON, 1951; THORSTEINSON, 1953, 1955). In the case of the present species, however, it is difficult to consider any nutritional stimulus in the process of host plant preference, because the gravid female selects soybean plants which are not the proper food for herself and the larva enters into a given pod to feed. The nutritional aspect in host preference is beyond the scope of this investigation. Therefore, attention will be directed mainly towards the relationship between the plant characters, a principal element of token stimuli, and oviposition behaviour relating to sensory physiology. The present paper deals with the results of field observations prior to the experimental analysis of the problem.

OVIPOSITION IN THE RANDOMIZED BLOCK FIELD

Material and method. In the years of 1952 and 1953, the randomized block field as shown in Figure 1 was placed in the field of Hokkaido University at Sapporo. In each of three rows within a plot, ten plants were grown at intervals of 25 cm. Two hairy varieties (Tokachi-Nagaha and Chusei-Hikarikuro) and two glabrous ones (Chusei-Hadaka and Nagaha-Hadaka No. 1) were chosen. On the 15th, 22nd and 30th of August and the 8th of September, three plants per variety were examined in detail for the number of eggs and the sites of eggs laid.

Result. In the varieties tested, the period of pod growing stage, extending from the end of July to the end of August, showed no difference among the varieties, and it coincided with that of the occurrence of the moth. This indicates that all the plants provide the same chance for oviposition. Consequently, assuming that each variety has an equal attractiveness to the gravid female, nearly identical numbers of eggs should be laid on all varieties. But the result shown in Figure 1 is adverse to this assumption. It showed clearly that much more eggs were laid on the hairy varieties (A, B) than in the glabrous ones (C, D), showing no remarkable differences both between the years and among the blocks. The number of eggs laid was 638 (A), 536 (B), 146 (C) and 123 (D) in the total of 1952 and 1953.

TABLE I

Percentage of eggs laid on various sites of the hairy and glabrous soybean varieties

Egg-laying	Variety			
sites	A	B	C	D
Leaf	< 1	< 1	0	0
Petiole	3	1	0	0
Stem	2	2	0	0
Stipule	7	5	70	76
Calyx	6	8	25	21
Pod stalk	4	4	0	0
Pod	77	80	5	3

In the number of eggs found at various sites of soybean plant given in Table I and Figure 1, there was a marked difference between the hairy varieties and the glabrous ones. In the hairy varieties, most of the eggs (i.e. 77—80 per cent) were found on the hairy pod. The next vegetative sites in order of preference for oviposition were the calyx and stipule. The eggs found on the stipule were always located on the outer surface. Very few eggs were also found on the leaf, petiole, stem and pod stalk. On the other hand, in the glabrous varieties the sites at which eggs were laid were restricted only to the stipule, calyx and pod. The eggs were found mostly on the inner surface of the stipule (70 per cent), though substantial numbers were found on the calyx (20 per cent). Moreover, the pod was the least attractive site for oviposition, contrasting with the hairy varieties.

The above observations suggest that the gravid moth shows some particular host plant preferences when ovipositing. This raises two problems which will be considered: (1) the preference between hairy and glabrous varieties, as shown by differences in egg numbers and sites (2) the preference for attractive sites for

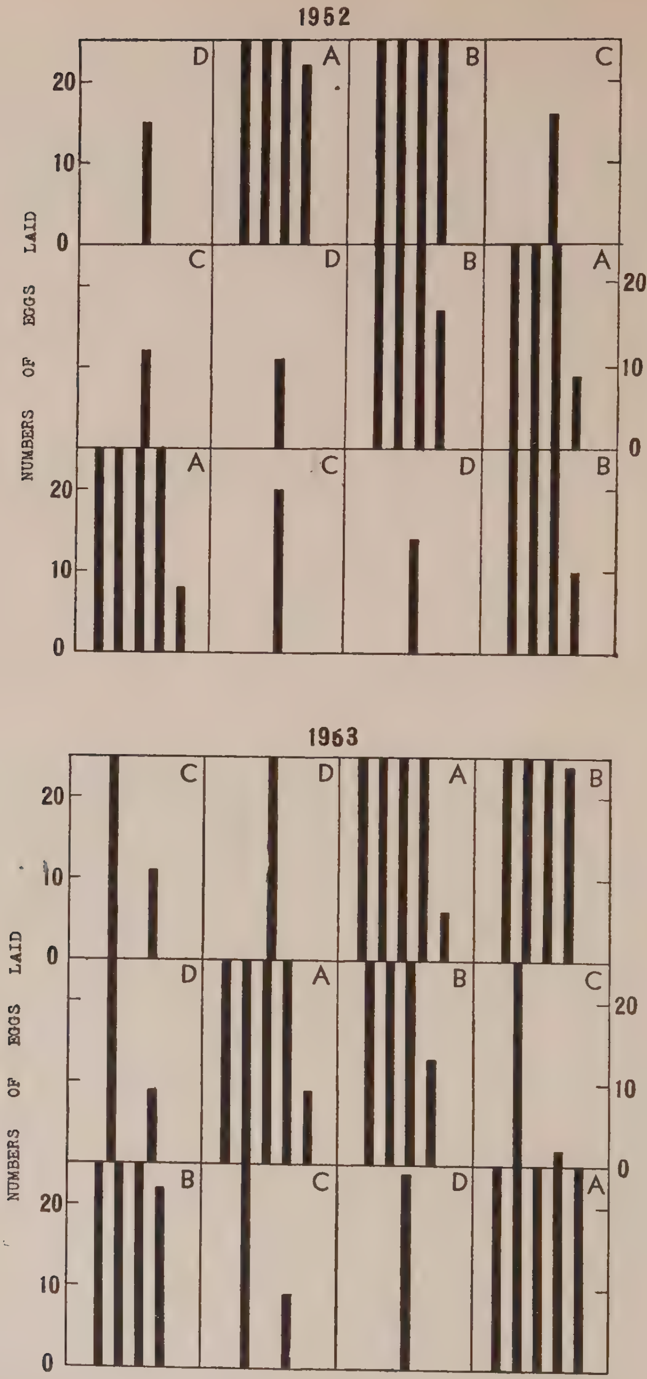


Figure 1. Numbers of eggs laid on the four soybean varieties (hairy A, B and glabrous C, D) in the randomized block field. The area of a plot was 4.125 m² (1.65 × 2.5 m)

egg-laying in soybean varieties. In field observations it may be difficult to determine the basis of these preferences.

OVIPOSITION IN AN ALTERNATELY PLANTED FIELD

Material and method. In 1954, two hairy soybean varieties, Tokachi-Nagaha (A) and Chusei-Hikarikuro (B), were sown alternately with two glabrous ones, Chusei-Hadaka (C) and Nagaha-Hadaka No. 1 (D), at intervals of 25 cm, in the layout shown below.

A C A C A C A C A C
D A D A D A D A D A
B C B C B C B C B C
D B D B D B D B D B

Four plots containing four varieties as shown above were provided in order to obtain more adequate data by replication. On the 15th of May, three seeds of each variety were sown close together at each position to avoid gaps if some seeds did not germinate. On the 20th of June the plants were thinned to leave one vigorous plant at each spot. The plants grew normally, the beginning of the flowering period being recorded on the last week of July. The first appearance of the moths was on the 29th of July. The main period of egg-laying activity was from the 15th of August to the 10th of September. During this period, the eggs laid on three plants per variety, which were marked at random from each plot, were counted at intervals of five days.

Result. The results are expressed by giving the totals for twelve plants per variety (3 plants × 4 plots) on each date of observation.

TABLE II

Number of eggs laid on the hairy and glabrous soybean varieties in the alternately planted field

Variety	Date of observation						Total
	15 Aug.	20 Aug.	25 Aug.	30 Aug.	5 Sept.	10 Sept.	
A	38	57	84	102	44	29	354
B	32	49	93	75	47	21	317
C	5	14	38	56	20	7	140
D	3	10	31	53	16	9	122

As shown in Table II, the number of eggs laid was clearly different between the two varietal groups. The eggs found on the hairy varieties (A, B) were more than twice as numerous as those found on the glabrous ones (C, D). In the former the favourite site for oviposition was the pod; no eggs were found on the glabrous pods. These results agree with the finding in the foregoing section. It is therefore concluded that the oviposition pattern of the species is not affected by the alternate plantation of the varieties of soybean which might cause disturbance of some factors attractive or repellent to the moth. In the hairy varieties, it is suggested that a greater egg-laying may be caused by the presence of hairy pods, because a large number of eggs are laid on the pod (see Table I). Then, a simple question arises: "Do hairy pods serve as an attractive site for oviposition?" To answer this question, the following experiment was conducted.

OVIPOSITION ON BEANS FROM WHICH THE PODS WERE REMOVED

Material and method. The soybean varieties used were Tokachi-Nagaha (A) and Nagaha-Hadaka No. 1 (D). The seeds were sown on the 15th of May, 1954, under the same plot-design as that illustrated in Figure 1. A block was divided into four plots in order to compare plants of A and D from which pods were removed with normally podded A and D. The podless soybean plants were obtained by plucking all the flower stalks throughout the flowering period, extending from the middle part of July to the end of August. On the 26th and 31st of August and the 6th of September, the eggs were counted on three plants per plot.

Result. The results are expressed by the total number of eggs per twelve plants (3 plants × 4 plots) on each date of observation.

TABLE III

Numbers of eggs laid on podded and podless soybean plants of the hairy and glabrous varieties

Variety	Treatment	Date of observation 26 Aug.	 31 Aug.	 6 Sept.	Total
A	Podded plot	97	114	31	242
A	Podless plot	20	28	12	60
D	Podded plot	36	49	7	92
D	Podless plot	42	55	10	107

As shown in Table III, the difference in the number of eggs laid in podded and podless plants was very great in the hairy variety, contrasting with the small difference in the glabrous one. The podless plants of the hairy variety (A) had far fewer eggs than the others. On the other hand, no considerable differences could be found between the two treatments in the glabrous variety (D). These results demonstrate clearly that the amount of oviposition is influenced considerably by the presence or absense of hairy pods, while it is not affected by glabrous pods at all. Thus the experiment suggests that the "hairy pod" provides an attractive or favourable site for oviposition.

A COMPARISON OF THE MICRO-CLIMATIC CONDITIONS IN THE HAIRY AND GLABROUS SOYBEAN FIELDS

A brief description of the microclimate may be useful for the consideration of the results mentioned in the preceding sections. In the hairy and glabrous soybean fields, the temperature and humidity at the level of the topmost pods between the rows were recorded from the 16th to the 31st of August in 1956, during the period that the moths were actively ovipositing. At the same time, the light intensity at various heights of a plant was measured by a luxmeter both under fine and cloudy weather conditions (see Fig. 2).

The mean temperatures in the hairy and glabrous soybean fields were 21.4° C

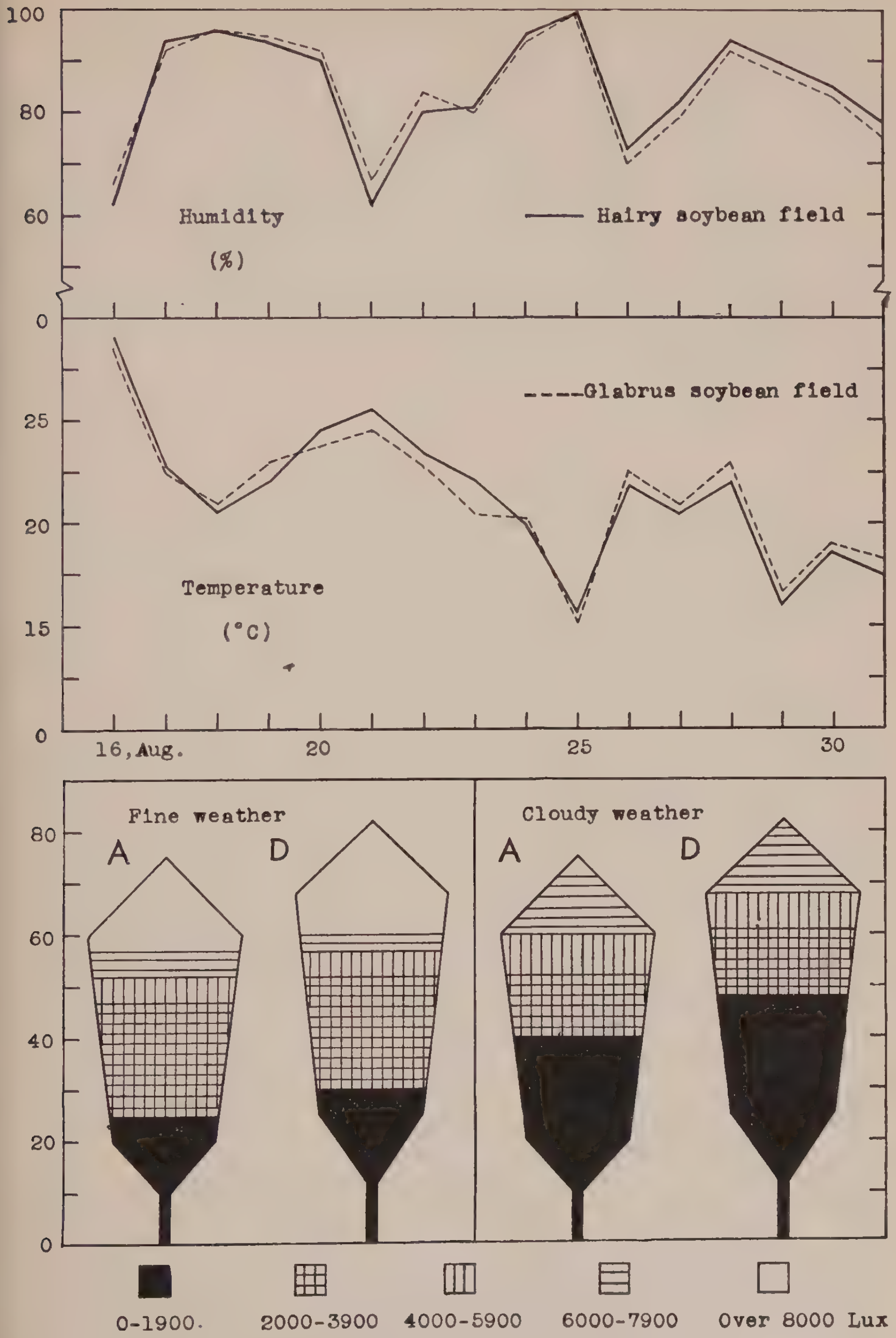


Figure 2. Comparison of the temperature, humidity and light intensity between the hairy and glabrous soybean fields.

and 21.3° C, with a range of 15.8—29.0° C and of 15.5—28.6° C respectively. The mean relative humidity in these fields was 83.8% and 84.8%, with a similar fluctuation as illustrated in Figure 2. These results indicate that, so far as temperature and humidity are concerned, no remarkable differences can be found between the hairy and glabrous soybean fields.

The light intensity near the soybean plant, which may cause a different pattern of oviposition by leading the moths to a different photo-stimulus, is of course changeable with weather, such as sunny or shady conditions, and also with the site of measurement. These differences are clear in Figure 2. For instance, the light intensity in fine weather was over 8000 lux above the topmost pods, while it decreased downwards on the plant. However, no *relative* difference between the hairy and glabrous plants could be found.

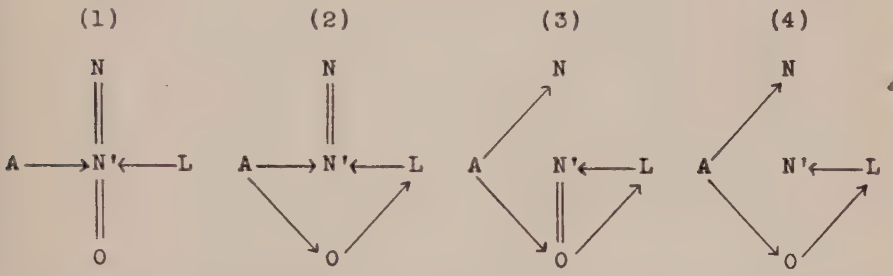
DISCUSSION

The subject of "host selection" or "food preference" being an important problem of entomology and the theme of the present investigation, some reference to general theory is called for. There are two main schools of thought on the subject. KENNEDY & BOOTH (1951), KENNEDY (1953, 1958) and FENNAH (1953) have claimed that host selection is a dual discrimination reaction that involves nutrition, particularly as it applies to tissue preference found in some species of Homoptera. This has been strongly disputed by DETHIER (1953, 1954) who has demonstrated that nutritionally unimportant token stimuli, such as attractants and repellents, are predominantly responsible for regulating feeding preference. He stated: "we should not expect *a priori* a close correlation between nutrition and plant preferences". This idea has also been supported by FRAENKEL (1953, 1958) and LIPKE & FRAENKEL (1956).

Apart from the validity of these theories, the present author considers that such conflicting opinions have been held, depending on whether attention has been directed towards "a dynamic process" or "a static analysis" and partly on the specific differences of the insects used. KENNEDY's work (1951—1958) on the food plant preference of aphids gives a dynamic rather than a static approach. This work is important, but at the same time work with phytophagous insects other than aphids must not be overlooked.

Aphids are insects in which adults and larvae are able to feed on the same kind of plants. Since their fecundity is strongly influenced by the food ingested, it may be possible to explain the relation between nutrition and host plant preferences. However, this is very difficult to invoke in the ovipositing female of some holometabolous insects, as has been pointed out by DETHIER (1954). For instance, most butterflies and moths are attracted to water, sugar or other sweet substances, probably for their feeding requirements, while they are also attracted to very different places for oviposition, usually a certain species or group of plants. Is there any connection between such preferences for feeding and oviposition? In this case, the meaning of "host plant preference" is, in the strict sense, not applied to "food preference", but merely to "oviposition site preference". When we consider the foregoing facts, it is apparent that the process of host plant preference is varied with the group of insects, and in order to avoid some confusion on the subject it is desirable to inquire first into the pattern of host plant preference.

Assuming that host plant preference in insects is concerned with adult (A) and its food for nutrition (N), oviposition site (O), larva (L) and its food for nutrition (N'), the following patterns will be considered.



The patterns are represented, for example, by most aphids and the Colorado beetle (*Leptinotarsa decemlineata* Say) in (1), most locusts in (2), boring or mining species of Lepidoptera in (3) and by the gypsy moth (*Lymantria dispar* L.) in (4) respectively. In the second and fourth patterns, the larvae are endowed with the abilities to migrate a good distance and to recognize suitable food, since their eggs are laid under the ground or in the vicinity of their food. In the third pattern, on the contrary, the larvae are never granted the opportunity to select their own food, since they are restricted by spatial factors to the food selected by the gravid female. The host plant preference for her progeny is largely determined by the abilities of the adult, and it should not be confused with the preference for her own food. The soybean pod borer belongs to the third pattern, since the oviposition is restricted on the host plant, *Glycine max* Merrill (soybean), and the larvae do not as a rule migrate from a given plant to a neighbouring plant (Kuwayama, 1938; Nishijima, 1954a). It is therefore obvious that the principal aspects of the host plant preference of the species are not related to nutrition, but to that of oviposition site preference, as discussed below.

It is well known that there is a considerable difference between hairy and glabrous soybean varieties in the egg number laid by the borer moth (Kuwayama, 1928; Uchida & Okada, 1937; Okada, 1948; Nishijima & Kurosawa, 1953; Nishijima, 1954a). This was also confirmed in this paper. Such a difference may raise the question whether this results from a different population of the moth crowding between the two groups of the varieties or a different micro-climatic condition in the fields. Indirect evidence on this question was given by the fact that such a difference was observed in the alternately planted field as well as in the randomized block field. Furthermore, the experimental fields, each representing a hairy variety and a glabrous one, showed similar climatic conditions (e.g. temperature, humidity and light). These facts indicate that the marked difference in number of eggs laid between the hairy and glabrous varieties is not a phenomenon caused by differences either of field climatic conditions or of moth population.

In the site of eggs laid on the soybean plant, there was a considerable difference between the hairy and glabrous varieties. In the hairy varieties the favourite site for oviposition was the pod while in the glabrous varieties it was not the pod (which showed the lowest percentage), but the inner surface of the stipule. Thsi tendency was found in each field experiment. It is therefore certain that such a clear difference in the site of eggs laid *between* the two groups of the varieties is unchangeable. On the other hand, *within* a variety, the rate of eggs found on various parts of soybean plant seems to be variable to some extent. As has been stated by KUWAYAMA (1928), the rate of eggs laid on the pod is, of course, to be changed or decreased when the pod bearing stage of plant does not coincide fully with the period of oviposition. In the case of the podless experiment, however, the number of eggs laid itself was greatly decreased by the complete removal of hairy pods. Thus a marked difference in the number of eggs laid between the hairy and glabrous varieties disappeared when the pods of the hairy variety were removed artificially. This suggests that the "hairy pod" is the most attractive site for oviposition, while the glabrous pod does not serve as such.

In conclusion, it is demonstrated that the host plant preference of the soybean pod borer shows two interesting aspects, namely, inter- and intra-varietal preferences. The experimental analysis of these aspects will be given in succeeding papers.

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ZUSAMMENFASSUNG

DIE WIRTSPFLANZENPRÄFERENZ DES SOJABOHNENBOHRERS, GRAPHOLITHA GLICINIVORELLA *MATSUMURA. 1. DIE EIABLAGE*

Die Wirtspflanzenpräferenz des Sojabohnenbohrers, eine wichtige Erscheinung hinsichtlich der Resistenz der Sojabohnenpflanze, wurde im Freiland unter Benutzung von je zwei Varietäten behaarter und unbehaarter Sojabohnen untersucht. Es zeigte sich, daß sowohl hinsichtlich der Anzahl als auch der Lokalisation der abgelegten Eier zwischen den haarigen und unbehaarten Varietäten der Bohnen ein beträchtlicher Unterschied besteht. Diese beiden Unterschiede wurden auch dann festgestellt, wenn die haarigen Varietäten im Wechsel mit den haarlosen gepflanzt worden waren. Als jedoch sämtliche Schoten aller Sojabohnenpflanzen künstlich entfernt wurden, konnte kein Unterschied in der Anzahl der abgelegten Eier mehr gefunden werden. Diese Behandlung verursachte bei den haarigen Varietäten eine übermäßige Verminderung der Eizahl, während dies bei den haarlosen nicht der Fall was.

Diese Beobachtungen zeigen offenbar, daß es hinsichtlich dieses Gegenstandes zwei Haupterscheinungen gibt, nähmlich Inter- und Intravarietätenpräferenz, und daß die erstere stark von der Anwesenheit der „haarigen Hülse" beeinflußt wird, die als die anziehendste Eiablagestelle zu gelten hat.

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THE EFFECT OF GAMMA RADIATION ON THE VIABILITY AND FERTILITY OF *LUCILIA SERICATA* MG. (DIPT.) IRRADIATED AS PUPAE

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The biological effects of gamma radiation on *Lucilia sericata* Mg. were studied by subjecting the pupae to a range of gamma doses from a Cobalt-60 source. Observations were made on the percentage subsequently emerging after each treatment, and their longevity. Fertility was measured by caging each sex with untreated flies of the opposite sex, the numbers of hatching egg clusters obtained providing an index of fertility of the treated component.

Percentage emergence, in pupae more than 3 days old at irradiation, was normal up to 12,000 rep, and reduced by about 24% after 24,000 rep. Adult longevity was progressively reduced by doses from 1,000 rep upwards, and was about 50% of the control value after 9,000 rep. The percentage viability of eggs from treated females decreased in the dosage range 0—2,000 rep without any decrease in total eggs produced, after 3,000 rep and above females failed to lay any eggs. Male fertility was about 3% after 3,000 rep; above 3,000 rep males were completely sterile. Morphologically normal sperm was produced by males at all doses up to 24,000 rep, and showed motility up to 6,000 rep at least.

Pupae irradiated before completion of a third of the total duration of the pupal stage failed to emerge after 3,000 rep; those irradiated after that stage emerged normally after 12,000 rep. There was evidence that the transition from susceptibility to resistance was abrupt and that the physiological age of the pupae corresponding to this resistance threshold was independent of velocity of pupal development.

BUSHLAND & HOPKINS (1953) showed that it was possible to produce sterilised viable adults of the screw-worm fly *Callitroga hominivorax* (Cqrl) by subjecting their pupae to gamma radiation. Flies so sterilised were used by Baumhover and his co-workers (BAUMHOVER, GRAHAM, BITTER, HOPKINS, NEW, DUDLEY & BUSHLAND 1955) as a means of eradicating an isolated population of the species. The method of insect control based on the release of sterilised individuals has been outlined and discussed by KNIPLING (1955) and LINDQUIST (1955).

This paper describes some biological effects of gamma irradiation of the pupal stage of *Lucilia sericata* Mg. (Dipt.). The ecology of this species would suggest it as a suitable organism with which to test further the practicability of this new technique of insect control.

METHODS

The test insects were bred from laboratory strains of *L. sericata.* Large cultures of larvae were obtained by pooling the first instars obtained from eggs of several females, and allowing them to feed on horseflesh contained in trays over silver sand. The fully-fed larvae were left for two days to empty the gut, after which they were recovered by sieving the sand, and allowed to pupate in batches of about 300 at a constant temperature of 25° C.

The larval cultures were cleared of pupae at least once a day, so that the age of pupae could be determined in all cases to within 24 hours. Except in one experiment, where the radiation susceptibility was being examined at different rates of pupal development, the pupae were retained at 25° C, at which temperature the duration of the pupal stage is about 8 days.

All the irradiation was done at Harwell or at the Wantage Radiation Laboratory, some 300 miles from this laboratory, involving a total transit period for the pupae of some 36 hours. The pupae were retained at constant temperature (usually 25° C) until the evening of the day before irradiation day, when they were dispatched by rail. Two methods of packing for transit were employed. For the first tests the batches, each of several hundred pupae, were packed in peat fibre in separate cylindrical cans of about 2-litre capacity. In Tests IV to VII, to decrease bulk and increase uniformity of γ-dosage, each test group consisted of about 300 pupae packed in single-pupae layers in 3 × 1 in. glass vials with a thin layer of cellulose wadding between each layer of pupae. The groups in each test were all packed within the same outer container, — a galvanised bin, well lagged within with dry wood-straw. This helped to cut down temperature variation during transit. In the test involving varying pupal temperature, the pupae travelled in fibre-board hay-boxes inside which the constant temperature was maintained by rubber water bottles. By this means the temperature variation was restricted to ± 2° C.

All the irradiation was done by the Technological Irradiation Group of Atomic Energy Research Establishment, Harwell. The gamma irradiation source was Cobalt-60. Tests I, II, III, and V were irradiated at a dose rate of 6.000 r. per hour and the remainder at 25,000 r. per hour.

Material was returned to the laboratory 18 to 20 hours after irradiation. Each test included a control group which accompanied the irradiated groups, but which received no irradiation. This is the "Travel Control" of Table I.

On return to the laboratory the material was returned to controlled temperature and humidity conditions to complete development. The percentage of treated flies which completed development as far as the eclosion of the imago was read as "% emergence". The fertility of any resulting imagines from each treatment group was tested by segregating the sexes at emergence and caging them with an equal number of untreated, newly emerged and virgin, laboratory-bred flies of the opposite sex. These test cages contained from 50 to 100 pairs, and were maintained for 3 to 4 weeks in an insectary with temperature and humidity controlled at 25° C and 60—65% R.H. Liver was placed in the cages periodically, and the numbers of fertile egg clusters (+), sterile clusters (—), and incompletely fertile clusters (±) laid on it were taken as the index of the fertility of the test cage. In some cases, however, actual counts were made of the percentage of the hatched eggs in each cluster. The sample of each test group which was used in the fertility tests provided information on longevity, (50% M, the number of days taken for 50% of the group to die), of the imagines resulting from irradiated pupae.

The treated flies are described by prefixing their dosage received in reps; e.g. a male receiving 6,000 rep is referred to as a 6,000 rep ♂.

RESULTS

Effect of varying γ-dosage

The results of four independent tests on the effects of a range of gamma doses administered to pupae of known age are given in Table I.

In none of the tests was there any measurable effect of the travel conditions, and the "travel control" values are consequently often used as a standard.

Apart from the first test, involving pupae less that 2—3 days old, the material survived doses up to 24,000 rep. Up to 12,000 rep, the emergence percentage was the same as that in the controls, while at 24,000 it was reduced by only some 24%. The variation in susceptibility to gamma radiation, in relation to the age of pupae at irradiation, will be examined in more detail later, but it is of interest to note here that 2—3 day old pupae succumbed to 3,000 rep, whereas 4 to 6 day old emerged normally after 4 times that dose. This effect, of increased susceptibility in younger pupae, is similar to that found by BUSHLAND & HOPKINS (1953) in the screw-worm fly, and POTTS (1958) in the tsetse fly.

There is evidence of reduction of longevity in the adults emerging from the irradiated pupae at all doses over the range of the doses tested. The differences between the tests in the actual value of the 50% M point for similar dosage have presumably been accentuated by the variation between tests in the pupal ages at irradiation. In Test IV there is an indication that increasing dosage increasingly shortened adult life.

The number of egg clusters laid in cages containing treated males with normal females (♂ / ♀) and normal males with treated females (♂ / ♀) provides an index of the fertility of the treated flies for comparison with the controls. At doses above 3,000 rep the males were effectively sterilised; at 3,000 rep the mates of treated males laid mainly (±) clusters with a fertility per cluster of about 3%. The sterilising effect on the females was more severe, since even at 3,000 rep their output was limited to a very occasional cluster usually non-viable (—), and above 3,000 rep they did not even lay eggs.

The fertility results of the fourth test are alternatively expressed in terms of the mean percentage of viable eggs per cluster. It will be seen that, as the dose increases, the percentage of viable eggs laid by the mates of treated males progressively decrease to a value of 2.67 at 3,000 rep. This would suggest an increasing percentage of affected sperms being produced by the treated males as the dose increased, above 3,000 rep all the sperms being affected. In the case of the treated females there is what appears to be a dual effect. As the dose increases from zero to 2,000 rep, oviproduction is constant, comparable numbers of eggs (15 normal sized clusters) being produced in each case, but the mean percentage viability of the eggs produced decreases from about 80—90 to 66, then to 27. Furthermore, as the dose increases to 3,000 rep, oviproduction is reduced to a total of 14 eggs representing about a tenth to a twentieth of a cluster. Beyond 3,000 rep, oviposition by treated females practically ceases. The three viable clusters obtained from the 5,000 rep group of this test may be evidence of a possible "tail-end" of residual fertility. They were, however, laid over time intervals which suggested that they were the product of a single female, so that the possibility of contamination cannot be entirely ruled out.

Test No.	Pupal age at irrad. (Days)	N	n		Basic Lab. Control	Travel Control 0 rep	3,000 rep	4,500 rep	6,000 rep	7,500 rep	9,000 rep	12,000 rep	24,000 rep
				Type of Fert. Test	♂/♀	**♂**/♀ ♂/**♀** **♂**/**♀**	**♂**/♀ ♂/**♀** **♂**/**♀**	**♂**/♀ ♂/**♀**	**♂**/♀ ♂/**♀** **♂**/**♀**	**♂**/♀ ♂/**♀**	**♂**/♀ ♂/**♀**	**♂**/♀ ♂/**♀** **♂**/**♀**	**♂**/♀ ♂/**♀** **♂**/**♀**
I	2—3	170	—	% Emergence	89.5	85	2.9		1.75			0	0
II	4—6	580	50 (3)	% Emergence	89,5	89	91		88.5			91	70
				50% M (days) **♂**/**♀**	>24/>24	>24/>24	>24/>24		21/21			18/>24	14/18
				Clusters +	15	21 9 19	3 0 0		0 0 0			0 0 0	0 0 0
				per ±	0	0 0 0	14 1 0		0 0 0			0 0 0	0 0 0
				cage —	1	2 0 1	1 1 3		8 0 0			12 0 0	6 0 0
III	5—6	400	50 (3)	% Emergence	90.5	90	84	90	90	84	89		
			♂/**♀** →	50% M (days)	>23/>23	20/>23	20/ >23	22/24	24/19	22/22	23/22		
				Clusters +	28	15 15	0 0	0 0	0 0	0 0	0 0		
				per ±	0	1 1	22 0	0 0	0 0	0 0	0 0		
				cage —	4	4 9	10 0	24 0	21 0	20 0	26 0		
				Mean Fert. %/Cluster			2.85						

					Basic Lab. Control	Travel Control 0 rep	1,000 rep	2,000 rep	3,000 rep	4,000 rep	5,000 rep	6,000 rep	7,500 rep	9,000 rep
				Type of Fert. Test	♂/♀	**♂**/**♀**	**♂**/♀ ♂/**♀**	**♂**/♀ ♂/**♀**	**♂**/♀ ♂/**♀**	**♂**/♀ ♂/**♀**	**♂**/♀ ♂/**♀**	**♂**/♀ ♂/**♀**	**♂**/♀ ♂/**♀**	**♂**/♀ ♂/**♀**
IV	6—7	300	75 (4)	% Emergence	90	96.7	94	97	95	96	97	90	93	90
				50 % M Pooled **♂**+**♀**	>40	40	34	37	35	24.5	24	23	23.5	18.5
				Clusters +	15	13	11 14	0 0	0 0	0 0	0 3	0 0	0 0	0 0
				per ±	0	2	3 1	10 15	9 1	0 0	0 0	0 0	0 0	0 0
				cage —	0	0	0 0	0 0	6 0	14 0	16 0	15 0	16 0	15 0
				Mean Fert. %/Cluster	90.9	81.1	58 66	8.7 27	2.67 4/14 eggs	0 0	0 0	0 0	0 0	0 0

N = number irradiated per group.

n = number of pairs caged in fertility test (figure in brackets — duration of cage test in weeks).

⚥ = untreated; **⚥** = treated.

50% M = Longevity as measured by time taken for 50% of individuals to die.

Examination was made of the state of the ovaries of a total of 58 females, including females which had been treated at 3, 6, 12, and 24 thousand rep. None showed normal ovarian development although all were over 2 weeks old, and had ingested protein. The normal pattern shown by 2 weeks old, protein-fed, females of the species is of a regular series of progressively more mature eggs within each ovariole, with correspondence between the ovarioles in the degree of development of successive follicles. In the material examined development was irregular within each ovariole and between ovarioles, with only a very few follicles beginning to form yolk, the majority appearing undeveloped or atrophied.

Sperm from treated males appeared to be morphologically normal in all the treatment groups including the 24,000 rep group. Tail movement was observed in expressed sperms of the 3,000 and 6,000 rep groups, but not in sperm expressed from males treated with 24,000 rep.

There was direct evidence, in one case, of transfer of sperm by a treated male; the spermatheca of a 6,000 rep female which had been caged with 6,000 rep males was observed to be full of sperm. Also, there are several instances (Table I) where viable eggs resulted from treated males and normal females, indicating mating between treated males and normal females, at least in the treatment groups up to 3,000 rep.

When the 3,000 rep males of Test II had died, their surviving untreated female mates were remated with the surviving untreated male mates of the 3,000 rep females. Similarly, the surviving (untreated) mates of the 6,000—24,000 rep groups were caged together. The females previously mated with the 3,000 rep males continued to lay ± clusters of about 3% viability, and those previously mated with 6,000—24,000 rep males continued to lay only clusters which were completely sterile. That is to say, the oviposition history of the females, after being offered a second mate, continued to reflect the fertility pattern of the first mate. A similar remating was carried out with the surviving females in Test III, using newly emerged virgin males as the second mates. In this case, however, some completely fertile clusters were obtained from the female ex-mates of treated males from each of the treatment groups. There is no clear evidence from the above whether mating with sterile males blocks the subsequent fertility of females once mated to them. Nor is the second case necessarily evidence that the females have mated more than once. It will be seen that, in the initial fertility tests, cage colonies of 50 pairs produced only 20 to 30 egg clusters per cage, which means that not all the females had laid eggs. In all of the control cages the total number of fertile egg clusters was much less than the number of females originally caged, suggesting that only a percentage had mated. It is possible that the viable eggs came from the re-mated Test III females which were still virgin when offered their second mate.

The progenies of 1,000 rep males and 1,000 rep females were bred through to the F_3 generation. The F_3 individuals appeared morphologically normal, and their percentage emergence and longevity were similar to those of normal laboratory-bred individuals.

Effect of varying pupal age at irradiation

A second series of experiments was undertaken to investigate the relationship between pupal age at irradiation and susceptibility to gamma radiation, the results of which are shown in Table II. In Tests V and VI the pupae were allowed to develop at 25° C and 100% R.H., throughout. The pupae were extracted from the larval cultures at one day intervals, and kept at 25° C. When a range of age groups, covering the whole pupation period, had been assembled they were despatched along with travel controls covering younger, older and mid pupal ages, for simultaneous irradiation at constant dose. Each group consisted of between 400 and 500 pupae.

The range of percentage emergence in the controls was from 70 to 90. In the two Tests V and VI, a small sample from each age group was killed at the time of irradiation to provide information on the stage of pupal development reached in each group. Thus Test VI was shown to include the following categories at irradiation:

8— 9 day old — emergence beginning, a few adults being irradiated;
9—10 day old — 50% emergence, half of group irradiated as adults;
10—11 day old — 100% emergence, all irradiated as adults.

Both tests included a group zero days old at irradiation, i.e. irradiated as larvae.

It will be seen from the Table II that emergence percentage was normal in pupae more than 2—3 days old. Pupae irradiated earlier than 2—3 days old did not emerge at all, while at 2—3 days old only occasional pupae survived. This effect was shown in both Tests V and VI, which it should be noted were at slightly different doses, and was repeated in Test VII in the group maintained at 25° C throughout.

The dead pupae in the groups irradiated earlier than 2—3 days old were examined macroscopically some weeks after their return to the laboratory, and showed evidence that development had occurred subsequent to irradiation. The material irradiated as larvae survived irradiation, and even pupated in large numbers; the pupae invariably formed without retraction of the larval body and were not viable. Those irradiated as adults also survived irradiation, but were completely sterile.

The more detailed aspects of the precise pupal stages associated with the acquisition of gamma tolerance, and the post-irradiation development are the subject of further study. The results of Tests V and VI show, however, clear evidence of the occurrence of a gamma-tolerance threshold stage in the development of the *L. sericata* pupae.

Results of longevity tests, made on Test VI material, also are shown on Table II. It will be seen that the longevity of adults increases with increasing pupal age at irradiation. The increased reduction of longevity in the oldest group over that of the two next oldest is probably a result of the confinement of emerged adults during post-irradiation transit.

Tolerance threshold age in relation to developmental velocity.

The general effect of pupal developmental velocity in relation to the effect of pupal age on gamma tolerance was studied. Pupae reared at 15° C, 25° C and 30° C were subjected to 7,000 rep at various ages as before. At these temperatures

TABLE II

Effect of uniform gamma radiation dose on emergence and adult longevity of pupae irradiated at a range of pupal ages, and at different velocities of pupal development

Test no.	V	VI		VII													
Dosage	6,000 rep	7,000 rep		7,000 rep													
Pre-irrad. temp.	25° C	25° C		15° C				25° C						30° C			
Post-irrad. temp.	25° C	25° C		15° C		30° C		15° C		25° C		30° C		15° C		30° C	
Pupal age at irrad.	% E	% E	50% M Days	% E	50% M Days	% E	50% M Days	% E	50% M Days	% E	50% M Days	% E	50% M Days	% E	50% M Days	% E	50% M Days
13—14 days	—	—	—	85.2	21	75.1	14	—	—	—	—	—	—	—	—	—	—
11—12 days	—	—	—	91	—	86.8	—	—	—	—	—	—	—	—	—	—	—
10—11 days	—	85**	26	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9—10 days	—	85	29	87.8	20	90	16½	—	—	—	—	—	—	—	—	—	—
8—9 days	91*	94*	29½	91.1	—	89.7	—	—	—	—	—	—	—	—	—	—	—
7—8 days	90	91	23½	88.8	12	87	15	89.8	20	90.8	17½	87.5	14½	—	—	—	—
6—7 days	93.5	70	19½	86.7	7	84.4	9	—	—	—	—	—	—	—	—	—	—
5—6 days	89.5	90	21	50.4	4½	41.9	4	84.5	14½	85.1	10	79.7	13	—	—	—	—
4—5 days	84	75	17	1.99	—	0.524	—	87	—	86.5	—	88.3	—	84	13	86	16
3—4 days	83.5	75	9½	0	—	0	—	57	8½	57.4	13	55.2	7	87.3	11½	83.8	9½
2—3 days	3.5	6	—	0	—	0	—	1.01	—	0.93	—	0	—	23.2	3	28	3
1—2 days	0	0	—	—	—	—	—	0	—	0	—	0	—	0	—	0	—
12—24 hours	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0—12 hours	0	0	—	—	—	—	—	—	—	--	—	—	—	—	—	—	—
Controls		90	34½	80	34	84	32	87.6		88		85.8		80	28	82	17

** Emergence completed.
* Emergence begun.

the total duration of the pupal stage is 14—16, 8—9, and 6 days respectively for 15°, 25°, and 30° C, roughly in the ratio 5 : 3 : 2. The effect of post-irradiation velocity of development on the tolerance-threshold age was also studied in the same test. This was done by subdividing each group immediately after irradiation and allowing some to continue developing at the original temperature and transferring some to another temperature. The manipulation of material after irradiation may be more simply explained by reference to Table II (Test VII): for example, each of the age groups developing at 25° C was divided into three parts, one part was allowed to complete development at 25° C, one was transferred to 15° C to complete development, and one to 30° C. The percentage emergence and longevity data are given in Table II.

Taking the results (Table II) of those groups which had developed throughout at the same temperature, it is seen that temperature in the range 15°—30° C has had no effect on the general pattern of tolerance in relation to physiological age. In each case the oldest pupae have emerged normally, the youngest have failed to emerge, and there is also in each series an intermediate group with reduced emergence percentage. In the slowest developing pupae (15° C) the "threshold" stage is ranged over two of the arbitrarily selected age groups and covers the pupal age range from 4—6 days, and in the 25° C series it covers the range from 2—4 days. The variation in each group of the pupal age at irradiation covers a full day, and is equal to the class interval. Biological variation in the material could well be equivalent to ± half a day's development; for example, the complete range of times taken to complete pupal development at 15° C was 5 days. Thus the interval of development time over which the resistance to gamma radiation was acquired could well be, and probably is, narrower than the time interval of one or two days suggested by the results shown in Table II. Even if the resistance were acquired "instantaneously" in the 15° C. Group at, say 5½ days, the 5—6 day old group would still contain 50% resistant and 50% susceptible individuals. Biological variation equal to + ½ a day's development would still give a few emergences in the earlier (4—5 days old) group.

These considerations tend to suggest the existence of some critical stage in pupal development whose achievement confers a degree of radiation resistance on the organism. The idea is further supported by the fact that physiological age at the threshold, as measured by the proportion of the total pupation time completed, does not seem to be dependent on the rate of development. The modal values for the total duration of pupation at 15° C, 25° C and 30° C were 15, 8½ and 6 days respectively. In the pupal groups developing throughout at 15° C the threshold occurred between the fourth and the sixth day, i.e. representing completion of from .27 to .4 of the total duration of pupation. At 25° C and 30° C the threshold occurred between stages of development represented by proportions of the total duration of pupation of from .24 to .47 and .3 to .5 respectively.

Finally, the results offer no evidence that the radiation effects were altered by altering the post-irradiation temperatures within the range 15° C—30° C. Within this range, the "recovery" period between irradiation and emergence, differed between groups identically reared up to irradiation and simultaneously irradiated at the same dose, by as much as a factor of two in some cases.

DISCUSSION

There is evidence from the results shown that gamma radiation damage may depend as much on the age of the organism at irradiation as on gamma dose. There is some evidence that the metamorphosing *L. sericata* pupae pass through a developmental stage when their radiation resistance increases. The results suggest that this stage is of short duration and may even be the occurrence of a histological event. MAVOR (1927) found the same pattern in the x-ray tolerance of *Drosophila* and suggested that the increase of resistance coincided with the completion of mitotic proliferation of the imaginal tissue masses. Mavor's hypothesis would imply that irradiation before the threshold age would prevent the formation of the tissue masses by interfering with mitosis, whereas, after the threshold, radiation would operate on existing cells which as they progressively matured would be progressively more resistant. The present results show the two effects expected on the basis of Mavor's hypothesis. None irradiated before the threshold emerge; the normal percentage emerge from pupae irradiated after the threshold. Also the resistance of the survivors, in terms of adult longevity, increases quantitatively as the age at which they were irradiated. There is, however, no histological evidence in MAVOR (1927) or here to support the hypothesis, and further work requires to be done on this aspect.

The results provide an indication of the lethal and sterilizing effects of a range of gamma dosage. Pupae more than one-third developed emerged normally after does up to 12,000 rep, with a 24% reduction in emergence percentage at 24,000. These results agree with those of BUSHLAND & HOPKINS (1953) who showed that screwworm fly pupae irradiated at up to 5,000 rep, when in their last two days of pupation, emerged normally. They also agree with Potts' findings for tsetse, which emerged normally after doses up to 12,000 rep if irradiated when more than one third developed.

There was some evidence that doses from 1,000 rep upwards reduced adult life and that this effect increased with increasing dose.

Sterilisation of *L. sericata* was achieved within the gamma dosage range 3,000—6,000 rep, which seems to be the effective range for many insects. CORNWELL, COOK & BULL (1957) found that seven out of thirteen species studied were completely sterilised with 6,000 rep or less, and that a reduction in fertility occurred in all at 3,000 rep and above. As earlier noted, *C. hominivorax* studied by Bushland & Hopkins also were sterilised in approximately the same range, 2,500 rep for males and 5,000 rep for females. In *L. sericata* however the relative resistance of the sexes to the sterilising effect of gamma radiation was the reverse of that for *C. hominivorax*. The males were slightly more resistant, requiring more than 3,000 rep for complete sterilisation whereas females were effectively sterilised at 3,000 rep. In this respect *L. sericata* resembles tsetse in which Potts showed fertility of the females to be reduced to 16% at only 3,000 rep and that of the males reduced to some 10—20% by 6,000 rep. There was some residual fertility in Potts' tsetse up to 12,000 rep. The present work failed to show any such effect of a long tail of residual fertility in *L. sericata*.

It is generally assumed that the first effects of gamma radiation are the inducation of dominant lethal mutations in otherwise functional gametes. Furthermore, the use of sterile males in population control prerequires such a situation, where the affected sperms in the gamete population must be able to compete effectively with the unaffected in fertilising the ova, and where no viable zygote can result from such a fusion. LEE (1958) was able to demonstrate that the sperm from drone bees, subjected to doses between 10,000 and 34,000 rep, were sterilised, but were capable of fertilising eggs which did not subsequently develop. Higher doses resulted in sperms which were incapable of fertilising eggs; all the eggs laid by queens mated to them, including the eggs laid in worker comb, developed into drones, that is they developed parthenogenetically.

In the present work there is evidence that *L. sericata* males were still producing apparently morphologically normal sperm in normal amounts after doses of up to 24,000 rep. Up to 6,000 rep at least the sperms were motile, and in one case sperms were observed in the spermatheca of a female mated with 6,000 rep males. The progressive decrease in male fertility over a dosage range 0—3,000 rep is probably therefore the result of a reduction in the percentage of fertile sperms being produced rather than reduction of total sperm capable of transfer to females.

In the case of *L. sericata* females irradiated with low doses, the first effect of increasing dose was to reduce progressively the percentage viability of eggs without affecting the total egg output. However, as dosage increased to 3,000 rep, egg output was very markedly reduced, and at dosages beyond 3,000 rep it ceased altogether. In females, induced lethal mutations alone might well bring about just such a situation, for the developing ovum represents a haploid organism in its own right. Furthermore, somatic damage, especially to the gut lining, which would interfere with digestion and absorption of ingested protein, could inhibit ovarian development in the species.

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RESUME

EFFET DE RADIATION GAMMA SUR LA VITALITÉ ET LA FERTILITÉ DE LUCILIA SERICATA *MG. (DIPT.) IRRADIÉ AU STADE DE PUPE*

Des pupes de *L. sericata* Mg. furent exposées à l'irradiation gamma d'une source de Cobalt-60 pour étudier le nombre d'adultes qui en émergeraient, leur longévité et leur fertilité.

Les individus irradiés durant le premier tiers de la vie nymphale succombent à 3.000 rep. Chez les individus irradiés durant les deux autres tiers du développement de la pupe, les effets suivants furent observés:

1) Le pourcentage d'émergence fut normal jusqu'à 12.000 rep.; à 24.000, l'émergence était réduite de 24%.

2) Les mâles et les femelles étaient stérilisés complètement aux doses supérieures à 3.000 rep. La fertilité des mâles traités, évaluée par le pourcentage d'œufs viables pondus par leurs conjointes, décroît progressivement lorsqu'on augmente le dosage d'une valeur d'environ 3% à partir de 3.000 rep.

Au-dessous de 3.000 rep. la production totale d'œufs chez les femelles traitées fut nor-

male, mais le pourcentage de viabilité décroît avec l'augmentation de la dose; au-dessus de 3.000 rep. les femelles ne pondent plus d'œufs.

3) La longévité des adultes fut d'autant plus réduite que la dose était accrue.

L'irradiation des individus à différents âges du stade nymphal mit en évidence que, dans le développement de la pupe, il y a un seuil situé environ au tiers de ce développement à partir duquel la résistance à l'irradiation est accrue. Les individus irradiés avant ce stade-seuil n'effectuent plus leur mue imaginale pour une action seulement de 3.000 rep.; après avoir passé ce stade l'émergence est normale même à 12.000 rep.

Le stade pupal auquel se présente ce seuil n'est pas fonction de la rapidité du développement nymphal, ni déterminé par l'altération du taux de développement résultant de l'irradiation. Il est suggéré que ce seuil correspond à un processus physiologique défini dans le développement nymphal.

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THE SETTLING OF TSETSE FLIES *GLOSSINA MORSITANS* WESTWOOD (DIPTERA, MUSCIDAE) ON CLOTH SCREENS

BY

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It has been found that tsetse flies prefer to settle on the darker of two screens but, contrary to expectation, they settle on the sunny side of a screen rather than on the shaded side. The attractiveness of a black and white striped screen diminished progressively as the width of the white stripes was increased but even narrow white stripes made a screen less attractive than an all-black control. This breaking up of the black screen was important since white adjacent to a black screen did not reduce its attractiveness.

More tsetse flies are caught on a fly-round (POTTS, 1930; NASH, 1930) and the female percentage is increased (Lloyd quoted by SWYNNERTON, 1933) if screens or bait animals are used. The colour of a screen is important with regard to the number of flies caught (LLOYD, 1935 for *G. swynnertoni*; JACK, 1939, for *G. morsitans*). Colour is also important in the settling of other biting flies (see discussion). The present investigation concerns the settling of tsetse flies on black, dark grey, light grey, white and on black and white screens.

The experiments were carried out during the last two weeks of August 1959 in a mopani woodland (*Colophospermum mopane* Kirk ex J. Leonard) to the N.E. of the Chitaki and Rekomitjie Rivers in the Zambesi Valley of Southern Rhodesia. The size of the experimental area was approximately three square miles. 3,188 tsetse flies were caught and 97.4% of these were *G. morsitans*, the rest being *G. pallidipes*. Experiments were usually completed before 1700 hrs. and *G. pallidipes* is most active after this time. This probably explains the low percentage of *G. pallidipes* in the total catch. 16.9% of the total flies caught were females.

METHODS

The catching party comprised two European scientific workers and two African assistants experienced in catching tsetse. To avoid "catching out" the tsetse population, a different route was followed for each part of an experiment. All screens were carried for 1,000 paces and this distance will be referred to as a transect. A stop was made every 50 paces for the collection and counting of flies.

In experiments with a single screen (6 × 3 feet) the attractiveness of the cloth on one side of the screen was compared with that of another cloth on the other side. All cloths had the same texture. The intensity of light reflected by the cloths (when a clear 100 W. glass bulb was one foot away and measured using an EEL Lightmaster photometer also one foot away) was: black = 2, dark grey = 6, light grey = 12 and white = 30 lumens per sq. ft. The screen was rotated every 50 paces to reduce the effect of differences in the environment on the two sides of the screen. To eliminate any "fly boy effect" (SMITH & RENNISON, 1958) each man collected the same number of times on the two sides of the screen and also on the sunny and shaded sides. The same precautions were taken in counting the flies settling on the screen. Differences in the catching ability of pairs of assistants are summarised in Table I. The difference between the total flies counted by A.M.S. (3,865) and R.B. (4,107), in all experiments, was statistically significant ($X^2 = 7.34$, $P = < 0.01$).

TABLE I

Comparison of the fly catching ability of pairs of assistants, A and B

A B	Flies per transect by A	Flies per transect by B	Number of times together
T and W	40.8	46.2	6
T and M	41.2	33.3	18
T and J	29.3	25.3	3
P and M	59.7	49.0	3

When catching a fly, other flies on the screen are disturbed. The counts of flies settling on the screen represent, therefore, choices for the screen and not the exact number of flies. This disturbance resulted in a lower percentage catch when a large number of flies were on the screen than when flies appeared one after another (Figure 1). Over the range covered, however, the number of flies caught is linearly related to the number of flies counted ($b = 0.214$). In view of this the findings will only be presented in terms of the number of flies counted, special mention being made in those experiments where the number of flies caught suggests a different result.

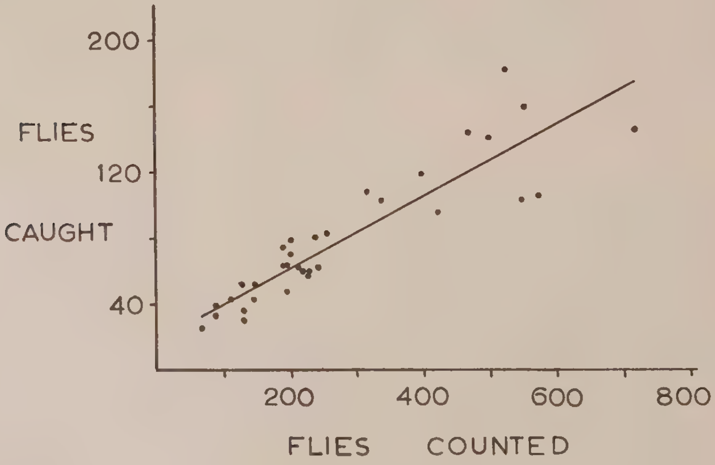

Figure 1. Comparing the number of flies caught on the screen with the number of flies counted as they settled on the screen

RESULTS

Experiment One

The first experiment was a comparison of darker with lighter shades. In Table II the number of flies landing on the two sides of the screen are shown. In every case more flies settled (and more were caught) on the darker side of the screen than on the lighter side.

TABLE II

The number of flies landing on the two sides of a cloth screen. The two sides differed only in shade : as shown in the table

	DARK GREY	LIGHT GREY	WHITE
BLACK	118 306 (72.2%)	43 149 (77.6%)	
DARK GREY		36 75 (67.6%)	22 169 (88.5%)
LIGHT GREY			24 64 (72.7%)

Experiment Two

In the second experiment, black, white, and black/white screens were compared. The black and white screen was 50% white, with white added as three vertical stripes dividing the black into four equal parts. The results are shown in Table III. The number of flies landing on the two sides of the screen was approximately the same when the sides were identical. When the two sides were different, most flies landed on the side with the most black in it. The same conclusions apply to the number of flies caught on the screen except that approximately the same number of flies were caught on the two sides of the screen when black was compared with black and white.

TABLE III

The number of flies landing on the two sides of a cloth screen. The two sides differed only in shade: as shown in the table

	BLACK	BLACK & WHITE	WHITE
BLACK	66 64	91 149 (62.1%)	23 220 (90.5%)
BLACK & WHITE		70 58	5 125 (96.2%)

Experiment Three

Many flies settle on a black screen but few settle on a white screen. In the third experiment the black side of a screen was used as a control and compared with the black and white side. The white was added as three vertical stripes or as three

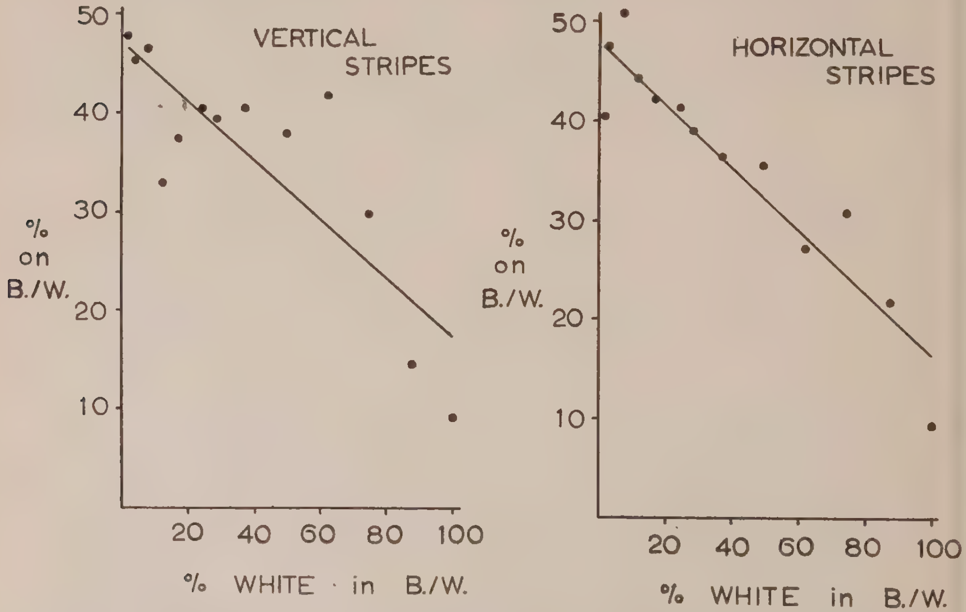

Figure 2. Showing the percentage of the total flies that settled on the black and white side of the screen, with different amounts of white (VERTICAL or HORIZONTAL stripes) in this screen. The black side of the screen was a control in each comparison

horizontal stripes, so as to divide the black into four equal parts. In both cases the percentage of flies counted (Figure 2) or caught on the black and white side decreased as the percentage of white in this side increased. The black screen was preferred even when the black and white screen contained only a small amount of white. The experiment revealed no difference in the behaviour towards vertically and horizontally striped screens. The regression coefficients for flies counted are: vertical stripes, $b = -.297$; horizontal stripes, $b = -.320$, the difference between these is not statistically significant, $t = 0.326$. Also the values of Y at $\overline{X} = 39.3$ are not statistically different, $t = 0.134$.

Experiment Four

In the previous experiment the amount of black was decreased as white stripes were added to the screen. An attempt was made, therefore, to compare two black screens of the same area, one of which had white added either all around or below or above the black. The black screens were 6 × 3 feet and the white was 6 inches wide in each case. The order of the screens in the procession was changed every 50 paces. The results are shown in Table IV. More flies landed on the black screen than on the black screen with white below it. The difference between the counts on the other screens was very small and in the opposite direction. The numbers of flies caught were similar in each case. Tsetse flies follow the catching party and, therefore, more flies might be expected to settle on the second screen than on the first. In each case a few more flies were caught on the second screen. The differences between counts are shown in Table IV.

TABLE IV

The number of flies landing on a black screen and on a black and white screen and on the first and second screens in procession

Experiment	Black	Black & White	First	Second
White all around Black	466	477	476	467
White below Black	296	229	252	273
White above Black	161	175	141	195

Experiment Five

Flies appear to settle equally on black screens of the same area, whether or not they have white parts adjacent to the black. Also, flies rarely settle on the white part of any black and white screen. The progressive reduction in the number of flies landing on a black and white screen as the amount of white in the screen increases could be due to the reduced area of black. Table V shows the results of a

TABLE V

The number of flies on screens of different area and in different positions in procession

	Black 6 × 3 ft	Black 2 ft 8 in × 4 ft 6 in	Black 3 × 2 ft	First	Second	Third
Flies Landing	77	72	48	58	59	80
Flies Caught	23	28	21	25	24	24

comparison of all-black screens of different area (18 square feet, 12 sq. ft., and 6 sq. ft.). The screens were carried in procession, their order being changed every 50 paces. The number of flies on the first, second and third screens is also shown in Table V. Fewer flies landed on the smallest screen than on the other two but the difference was not big enough to account for the results with black/white screens. Also most flies landed on the screen which was carried third in the procession. Neither of these differences was revealed in the number of flies caught.

Flies Settling in Direct Sunlight

In all experiments in which the tsetse had a choice, the darker side of the screen was preferred. It might be expected from this that they would also rest on the shaded rather than on the sunny side of a screen. This was not so. In experiment two, for example, the sunny sides of the screen were preferred whether they were black, black and white or white (Figure 3). Considering all experiments with a single screen and ignoring all other differences between its two sides, it was found that the tsetse preferred the sunny side: 153.4 flies per transect were counted on the sunny side, and 126.7 on the shaded side (t = 3.9; P = 0.001). The black control screen was preferred when it was in the sun to when it was in the shade (t = 7.4; P = 0.001).

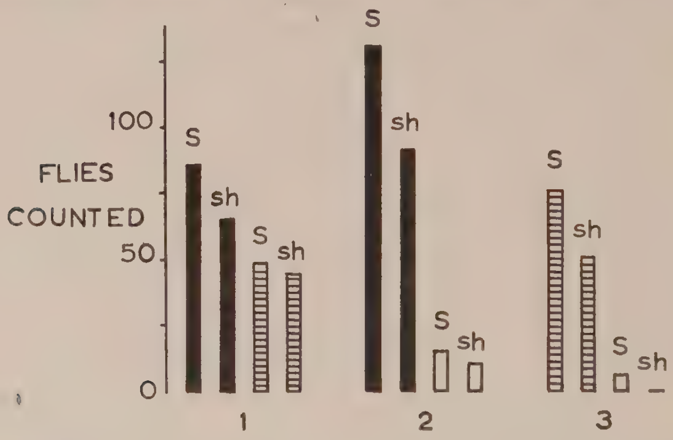

Figure 3. The number of flies counted on the sunny (S) and shaded (sh.) sides of each screen are shown. The screens were compared in pairs as follows: 1. Black with B/W. 2. Black with White and 3. B/W with White.

DISCUSSION

Several workers have noted that tsetse flies settle on a darker rather than on a lighter surface (MALDONADO, 1910; SIMPSON, 1911; SWYNNERTON, 1933; LLOYD, 1935 and JACK, 1939). Many of the tsetse fly traps described by SWYNNERTON (1933) were designed on the assumption that the flies prefer to settle on a darker surface. JACK (1939) caught most flies in traps covered with a dark material. Certain other biting Diptera show a preference for darker surfaces: the mosquitoes *Culex* (KO, 1925) and *Aedes* (KO, 1925; GJULLIN, 1947 and BROWN, 1951); the black fly *Simulium venustum* Say (DAVIES, 1951); the tabanids *Haematopota pluvialis* L. (PORTCHINSKY, 1915) and *Haematopota insidiatrix* Austen (BARRASS, 1959). *Lyperosia* (*Haematobia*) (Muscidae) prefers dark coloured cows (HAMMER, 1941) and the black parts of black and white cows (HAMMER, loc. cit. and THOMSEN, 1938). On the other hand KRIJGSMAN & WINDRED (1930) showed a preference for lighter colours in this insect. Among non-biting Diptera, WATERHOUSE (1948) found that *Musca domestica* L. preferred to rest on darker surfaces when offered a choice.

The identification by Weitz (WEITZ & JACKSON, 1955; WEITZ & GLASGOW, 1956) of blood from the gut of tsetse flies makes it clear that not all of the available game animals are fed on to the same extent. The colour of an animal might be of some importance in determining host preference (MOGGRIDGE, 1936; JACK, 1939) and a black animal may be preferred to a black and white one (HARRIS, 1923; HARRISON, 1940). Tsetse flies are also said to settle more on the black part of an animal's coat than on the white part (CURSON, 1924; FULLER & MOSSOP, 1929). These few records suggest that the behaviour of flies towards host animals might be in some ways similar to their behaviour towards screens.

It is not clear, however, why tsetse flies settle on screens and bait animals. Many of the flies attracted to a catching party are not hungry (BUXTON, 1955) so that an explanation cannot be found simply in terms of host location. Male flies are caught in far greater numbers than the females and attempted matings are often observed on the screen. It seems probable that a moving object represents a feeding situation for both sexes and also a place where males can find females (LLOYD, 1912). When screens are used, more flies are caught (LLOYD, 1935). This could be because more flies are attracted or because they are easier to see and to catch. Flies following a catching party settle when the party stops moving (some settle before this). The flies can then be collected off the members of the party, off the ground or off the nearby vegetation. A screen may simply provide a suitable surface for this settling behaviour.

It has been shown that flies prefer to settle on a darker screen especially if this is in the sunlight. The behaviour of these "active" flies is thus quite different from that of "resting" flies which are almost invariably found on the shaded side of a tree (NASH & DAVEY, 1951) or on the underside of a branch (ISHERWOOD, 1957). The settling in the sunlight is conducive to rapid water loss and was, therefore, quite unexpected. Previously, it was possible to argue that flies settled on a black screen because it "represented" a patch of shade (JACK, 1939) but this explanation now seems unlikely.

Comparison of screens of different shades indicates that the screens reflecting least light are preferred for the settling behaviour. In contrast each screen is most attractive when it reflects most light: that is, when it is in the sunlight.

The black and white striped screens are least attractive when the white stripes are widest. Even narrow white stripes reduced the suitability of a screen as a resting surface. In contrast, white adjacent to a black screen had no effect on the number of flies settling on this screen. The breaking up of the black surface seems to be the critical factor in this avoidance of black and white.

I wish to thank Mr. J. Ford, Director of Tsetse and Trypanosomiasis Control and Reclamation in Southern Rhodesia, for permission to use the facilities of the Rekomitjie Research Station. I was assisted throughout the work by Mr. A. M. Simmonds (Field Officer in Charge of the Research Station) and to him, especially, I owe my thanks. I also thank Professor E. B. Edney and Dr. E. Bursell for reading the manuscripts to this paper and for their comments. The German summary was prepared by Professor B. H. Rasmussen.

ZUSAMMENFASSUNG

DIE WAHL VON STOFFSCHIRMEN VERSCHIEDENER HELLIGKEIT DURCH SICH SETZENDE TSETSEFLIEGEN, GLOSSINA MORSITANS *WESTWOOD (DIPTERA, MUSCIDAE)*

Die Wahl von verschieden hellen Stoffschirmen durch sich setzende Tsetsefliegen wurde im Freien beobachtet. Schwarze, dunkelgraue, hellgraue und weisse Schirme wurden verglichen; in allen Fällen wurde der dunklere Schirm vorgezogen. In ähnlicher Weise setzte sich eine grössere Anzahl von Fliegen auf einen schwarzen Schirm statt auf einen weissen desselben Formats, während ein schwarz-weiss gestreifter Schirm an Anziehungskraft um so mehr einbüsste, je breiter die weissen Streifen gemacht wurden. Fast alle Fliegen setzten sich auf die schwarzen Teile eines schwarz-weiss gestreiften Schirmes. Ein weisser Streifen neben einem schwarzen Schirm konnte jedoch die Anziehungskraft des letzteren nicht vermindern. Gegen alle Erwartungen zogen die sich setzenden Fliegen aber die sonnige Seite des Schirmes der schattigen vor.

Es wird auf frühere Experimente hingewiesen, die Tsetsefliegen wie auch andere stechende Fliegen betreffen. Die Frage, warum Tsetsefliegen sich an Schirme setzen, wird erörtert und die Resultate der Arbeiten, über die hier berichtet wird, werden besprochen.

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THE INTERACTION BETWEEN ORGANOPHOSPHORUS INSECTICIDES AND ESTERASES IN HOMOGENATES OF ORGANOPHOSPHATE SUSCEPTIBLE AND RESISTANT HOUSEFLIES

BY

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Housefly homogenates contain different esterases, which compete for added organophosphates. Among these the cholinesterase and an ali-esterase rank first. If small amounts of organophosphate are added, the final result of the competition depends on the amounts of the esterases present and their reaction rates. The bimolecular rate constants (Table I) show that the ali-esterase is a powerful competitor of the cholinesterase for DDVP, paraoxon and diazoxon. Accordingly it was found that removal of the ali-esterase by denaturation (AD-treatment) strongly enhances the inhibition of the cholinesterase by these three compounds (Tables II, III and IV; Figs 1, 2 and 3), but not by tetram and QAT.

In homogenates of organophosphate resistant flies, which as previously shown contain much less ali-esterase, cholinesterase inhibition was generally much stronger than in "susceptible homogenates" (Table IV : Figs 1, 2 and 3). It seemed difficult to reconcile these findings with previous observations showing that low ali-esterase activity and resistance are closely connected. Further studies, however, showed that resistant flies contain a modified ali-esterase which has the capacity of degrading the organophosphates (P = O compounds) to which resistance has developed. The change of the ali-esterase (in susceptible flies) into a "phosphatase" (the modified enzyme in resistant flies) is due to a single gene mutation, which in the two resistant strains investigated is also responsible for the resistance. In each of these two strains the phosphatase shows a very high affinity for its substrates-paraoxon and diazoxon.

The hydrolytic action of esterases on their substrates has been shown to occur in two steps: 1) an acylation of the enzyme and 2) the hydrolysis of the acylation product. The reaction between esterases and organophosphates may likewise be depicted as occurring in two steps: 1) a phosphorylation of the enzyme and 2) the hydrolysis of the phosphorylated enzyme (see DIXON & WEBB, 1958). In many cases the latter reaction is extremely slow, i.e. the enzyme is very slowly regenerated and the organophosphates are considered as inhibitors. Whether an organophosphate is to be considered as a substrate or as an inhibitor thus largely depends on the rate of the dephosphorylation reaction, which in turn is dependent on the nature of the enzyme and on that of the reacting compound.

Irrespective of the hydrolytic or inhibitory nature of the reaction between enzyme and organophosphate, this reaction will induce the disappearance of free organophosphate. Crude homogenates of animal tissues may contain several esterases, which either hydrolyse organophosphates (i.e. phosphatases) or are inhibited by these compounds. If an organophosphate is added to such a homogenate the different esterases will be engaged in competition for the molecules of this compound. Consequently the inhibition which any particular esterase undergoes will not only depend on the characteristics of its reaction with the organophosphate but also on the presence of other esterases and phosphatases in the homogenate.

The present study was undertaken to investigate this in homogenates of houseflies. The ultimate purpose was to get a better insight into the molecular toxicology of organophosphate poisoning of insects, including both the intoxication and the detoxication aspect. Organophosphate resistant and susceptible flies were compared since they were found to differ markedly not only in sensitivity but also in esterase activity (VAN ASPEREN & OPPENOORTH 1959).

MATERIALS AND METHODS

Strains of houseflies. The following three strains of *Musca domestica* L. were used:

S Susceptible. Kept in the laboratory for many years.

D Collected in Denmark after the use of parathion and kept under light parathion pressure. Identical with the Danish strain used by BUSVINE (1959).

C Collected in Italy after the use of diazinon and kept under light diazinon pressure. Identical with the Italian strain used by BUSVINE (1959).

The cross resistance patterns of the strains D and C have been described by BUSVINE (1959) and by OPPENOORTH (1959). The resistance factors obtained by a contact method (LD 50 of resistant strain divided by LD 50 of strain S) for strains D and C are 10 and 30 for parathion, 7 and 20 for paraoxon, 7 and 35 for diazinon, 7 and 50 for diazoxon, and 1.5 and 2 for DDVP.

The flies were reared on a milkpowder - yeast diet at 25° C and about 60% R.H. and used 0—3 days after emergence.

Homogenates were made in ice-cold 3.5% NaCl-solution, using a small type Waring blendor, and kept at about 0° C till use.

Measurement of esterase activity was performed by the Warburg manometric method. The final reaction medium contained 3% NaCl and 0.025 M $NaHCO_3$ and was gassed with 95% N_2—5% CO_2 mixture, pH was 7.5. Cholinesterase activity was measured at 37° C using acetylcholine 1.5×10^{-2} M as a substrate and ali-esterase activity at 27° C using methylbutyrate 5×10^{-2} M or a phenylbutyrate-emulsion as substrates.

The organophosphates used were:

DDVP = O,O-dimethyl O-2,2 dichlorovinyl phosphate
paraoxon = O,O-diethyl O-p-nitrophenyl phosphate
diazoxon = O,O-diethyl O-(2 isopropyl-4-methyl-6-pyrimidinyl) phosphate
tetram = O,O-diethyl-S-2 diethylaminoethyl phosphorothiolate oxalate
QAT* = O-ethyl-S-2 triethylammonium ethylphosphonothiolate iodide.

Measurement of inhibition. Samples of the homogenate under investigation were exposed to different concentrations of inhibitor for a certain period of time (incubation period), after which the substrate was added and esterase activity measured. Comparison of the activities found with that of an uninhibited sample permitted calculating the percentage inhibition. From the inhibition values the I_{50}, i.e. the concentration giving 50% inhibition of the esterase activity could be calculated. Unless otherwise stated, the incubation period was taken so long that no further changes of inhibition were occurring. This "infinite incubation period" was determined in separate experiments.

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*) Quaternary phosphonothiolate Analogue of Tetram.

Measurement of excess inhibitor. If the addition of an organophosphate resulted in incomplete inhibition of the esterase activity after an infinite incubation period it was concluded that no free inhibitor was present at that time. When, however, inhibition was complete, free inhibitor was more or less likely to be present. Information about the amount of free inhibitor present was then obtained by adding known amounts of the homogenate to a freshly prepared homogenate of housefly heads and measuring the resulting inhibition of the cholinesterase activity of the latter homogenate.

The rate of the inhibitory reaction was studied by incubating the homogenates with the organophosphates for different periods of time and measuring the resulting inhibition of esterase activity. Plotting inhibition (ordinate) versus incubation time (abscissa) nearly always gave lines that after an initial rapid increase levelled off quickly. This behaviour may be explained by considering the inhibitory reaction in some more detail.

$$\mathrm{EH} + \mathrm{PX} \rightarrow \mathrm{EP} + \mathrm{HX}$$

where EH = the esterase studied
PX = the inhibitor used
EP = the phosphorylated enzyme
HX = the alcoholic, phenolic or acidic reaction product.

The rate of the reaction will be given by:

$$v = k \times [\mathrm{EH}] \times [\mathrm{PX}] \qquad (1)$$

where k = the bimolecular rate constant of the inhibitory reaction.

It was necessary to use low inhibitor concentrations in order to keep the reaction velocity at a measurable level. Consequently [PX] rapidly decreased during the reaction, especially so because it was, in most cases studied, also involved in other reactions, e.g. with other esterases present in the homogenate. The bimolecular rate constant was, therefore, calculated from the initial inhibition rate, since during this first period [PX] could be taken equal to the total inhibitor concentration ([I]). If v is expressed as the fraction (α) of the initial enzyme concentration $[\mathrm{EH}]_0$ that is inhibited during a short initial period t, equation (1) may be substituted by

$$\frac{\alpha}{t} \times [\mathrm{EH}]_0 = k \times [\mathrm{EH}]_0 \times [\mathrm{I}]$$

$$\text{or} \quad k = \frac{\alpha}{[\mathrm{I}] \times t}$$

Since many approximations had to be made for the actual calculations, the rate constants obtained should be considered as rough values.

Inactivation of ali-esterases. Theoretical considerations (see introduction) led to the assumption that the inhibition of some esterase in a homogenate will depend on the presence of other esterases. Since ali-esterases, which show high activity to a number of substrates in freshly prepared house fly homogenates, are readily inactivated in slightly alkaline media (VAN ASPEREN 1959), a useful approach to testing the above hypothesis could be made. Inactivation of ali-esterases was achieved by adding 0.025 M $NaHCO_3$ to the homogenate but omitting the introduction of the N_2—CO_2 gas mixture. The pH in the homogenates then rose to about 8.3. Keeping the homogenates under these conditions for ½—1 hrs or longer at 37° C resulted in a sharp decrease of ali-esterase activity. In one experiment the inactivation amounted to 85% after one hour. The cholinesterase activity was not appreciably altered by this treatment and inhibition of this enzyme by organophosphates could thus be measured with and without inactivation of the ali-esterases.

The inactivation of ali-esterases showed a very high temperature coefficient (Q_{10} = 10 approx.) pointing to a denaturation process. The above treatment will, therefore, be indicated as "AD-treatment" (Aliesterase-Denaturation-treatment).

RESULTS

Experiments were performed in order to obtain information on: 1) the rates of the inhibitory reactions of organophosphates with different esterases 2) the inhibition of esterases by organophosphates in homogenates that differed widely with respect to their esterase content and 3) the presence in the homogenates of phosphatases or organophosphate binding materials other than the esterases.

1. *The rates of the inhibitory reactions*

Whole fly homogenates (Strain S) were incubated with the organophosphates at 27° C for different periods of time and the bimolecular rate constants calculated as described in the section on methods. The results are shown in Table I.

TABLE I

Bimolecular rate constants of the inhibitory reactions at 27° C
(Litres. $Moles^{-1}$. $Min.^{-1}$).

	Cholinesterase (Substr. acetylcholine 1.5×10^{-2} M)	Ali-esterase (Substr. phenylbutyrate emulsion)
DDVP	8×10^{6}	1×10^{7}
paraoxon	5×10^{6}	8×10^{7}
diazoxon	4×10^{7}	4×10^{7}
tetram	4×10^{7}	6×10^{5}
QAT	5×10^{8}	4×10^{5}

The data indicate a distinct preference of paraoxon and a weak preference of DDVP for the ali-esterase. Tetram and QAT showed a striking preference for the cholinesterase. Diazoxon takes a neutral position.

2A. *The inhibition of cholinesterase activity in different homogenates*

a. Inhibition in fly head homogenates (Strain S) before and after AD-treatment. The homogenates were incubated with different concentrations of the organophosphate inhibitors and the I_{50}-values (concentrations giving 50% inhibition) obtained by interpolation. The results are shown in Table II.

TABLE II

Inhibition of fly head cholinesterase by organophosphates

Strain S; 2.5 heads/flask; T = 37° C; Mean act. of fresh homogenates ∽ 150 μl CO_2/30 min. Mean act. after AD-treatment ∽ 125 μl CO_2/30 min.

	I_{50} (Moles/Litre)	
	fresh homogenates	after 1 hr AD-treatment
DDVP	1.0×10^{-9}	0.7×10^{-9}
paraoxon	2.7×10^{-9}	1.6×10^{-9}
diazoxon	1.2×10^{-9}	0.5×10^{-9}
tetram	1.2×10^{-9}	1.2×10^{-9}
QAT	0.9×10^{-9}	0.8×10^{-9}

AD-treatment thus lowers the I_{50}, i.e. increases the "sensitivity" of the cholinesterase, for DDVP, paraoxon and diazoxon, whereas it has no effect on inhibition by tetram and QAT.

The effect of AD-treatment is thought to be due to interference with the organophosphate binding capacity of the ali-esterase. Therefore, similar and even more distinct effects would be expected in experiments on whole fly homogenates since only 20% of the ali-esterase is present in the heads (VAN ASPEREN, 1959).

b. inhibition in whole fly homogenates (Strain S) before and after AD-treatment. Cholinesterase inhibition was determined in fresh homogenates and in homogenates that had undergone AD-treatment for different periods.

TABLE III

Percentage cholinesterase inhibition in fresh and AD-treated whole fly homogenates of strain S

An "infinite incubation period" was allowed for DDVP, diazoxon, tetram and QAT. Paraoxon-inhibition was measured after 1½ hr incubation without having reached its final value

			AD-treatment for		
		0 hr	1 hr	2 hr	3 hr
DDVP	10^{-9}M	2	41	47	49
	2×10^{-9} M	3	79	79	—
Paraoxon	4×10^{-9} M	3	29	64	73
	8×10^{-9} M	53	88	95	—
Diazoxon	2×10^{-9} M	12	73	89	—
	4×10^{-9} M	40	100	98	—
Tetram	10^{-9} M	46	52	—	—
	2×10^{-9} M	72	83	—	—
QAT	5×10^{-10} M	46	47	—	—
	10^{-9} M	79	78	—	—

Table III shows that AD-treatment considerably enhances the inhibition brought about by DDVP, paraoxon and diazoxon whereas it has no appreciable effect on inhibition by tetram and QAT.

Further experiments on S-fly homogenates were performed, in which the inhibitions caused by five different concentrations of DDVP, paraoxon and diazoxon in both fresh and AD-treated homogenates were determined. From the data obtained the concentration-inhibition curves could be drawn (Figs 1, 2 and 3) and the I_{50}-values calculated (Table IV). AD-treatment caused a distinct shift of the concentration-inhibition curves to the left with a consequent decrease of the I_{50} values. The effect was more pronounced with DDVP and paraoxon than with diazoxon.

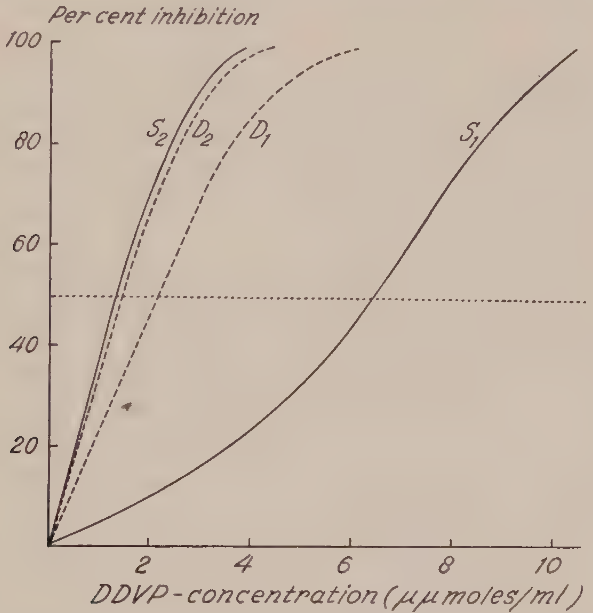

Fig. 1. Concentration-inhibition curves for cholinesterase and DDVP.
1.5 flies/flask; T = 37° C; AD-treatment during 1 hr.
S_1 = curve for fresh homogenates of susceptible Flies (Strain S)
S_2 = curve for AD-treated homogenates of susceptible Flies (Strain S)
D_1 = curve for fresh homogenates of resistant Flies (Strain D)
D_2 = curve for AD-treated homogenates of resistant Flies (Strain D)
1 μμ mole/ml = 10^{-12} mole/ml = 10^{-9} mole/litre.

c. *inhibition in whole fly homogenates of resistant flies (Strain D) before and after AD-treatment.* In the previous sections it was shown that AD-treatment inactivates the ali-esterase and increases the inhibition of the cholinesterase by organophosphates. The latter effect is thought to be due to the decrease in the amount of native ali-esterase. This hypothesis was further tested by experiments on whole fly homogenates of Strain D, which show only 20% of the ali-esterase activity (to methylbutyrate) of S-homogenates and, therefore, are more or less

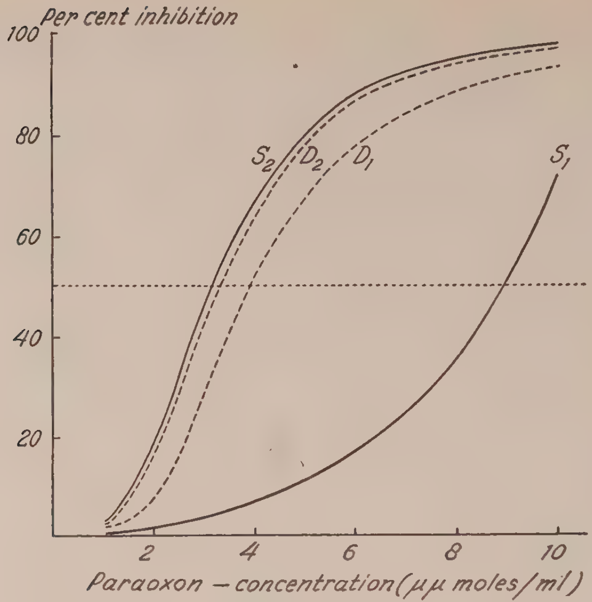

Fig. 2. Concentration-inhibition curves for cholinesterase and paraoxon. Explanation: see fig. 1.

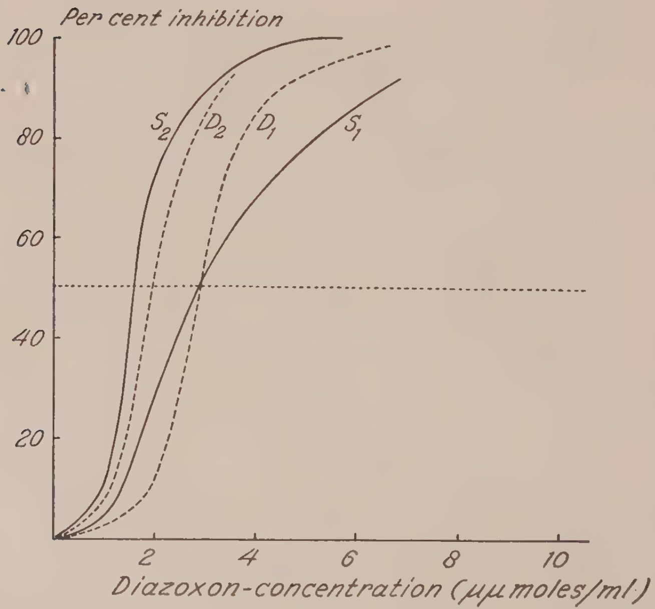

Fig. 3. Concentration-inhibition curves for cholinesterase and diazoxon. Explanation: see fig. 1.

TABLE IV

Cholinesterase inhibition in fresh and AD-treated whole fly homogenates
1.5 flies/flask; T = 37° C.

	I_{50} (Moles/Litre)					
	DDVP		Paraoxon		Diazoxon	
	S	D	S	D	S	D
Fresh homogenates	6.5×10^{-9}	2×10^{-9}	9×10^{-9}	4×10^{-9}	4×10^{-9}	3×10^{-9}
After AD-treatment (1 hr)	1.5×10^{-9}	1.5×10^{-9}	3×10^{-9}	3×10^{-9}	1.5×10^{-9}	2×10^{-9}

comparable to AD-treated S-homogenates. The results of the experiments (Figs. 1, 2, 3 and Table IV) were in full agreement with this assumption as far as DDVP and paraoxon were concerned; the data obtained with diazoxon were less clear. The inhibition of fresh D-homogenates was only slightly lower than that of AD-treated D-homogenates, which was about equal to that of AD-treated S-homogenates.

2B. *The inhibition of ali-esterase activity in different homogenates*

This was not extensively studied. Therefore, we shall confine ourselves to some observations that may be important for the present subject.

a. The ali-esterase activity towards methylbutyrate (0.05 M) in S-fly homogenates, which amounted to about 350 μl CO_2/fly at 27° C, was found to be strongly inhibited by DDVP, paraoxon and diazoxon but only weakly by tetram and QAT. The I_{50}-values found were 3×10^{-9} M for DDVP, 3×10^{-9} M for paraoxon and 3.5×10^{-9} M for diazoxon. However, these values cannot be directly compared with those obtained for cholinesterase inhibition, since only 1 fly/flask was used in the ali-esterase experiments whereas 1.5 flies/flask was used in the cholinesterase experiments. Substantial experimental evidence was obtained that the I_{50} values are strictly proportional with the homogenate concentrations, so that the values for the latter homogenate concentration (1.5 flies/flask) may be calculated to be approximately 4.5×10^{-9} M for DDVP, 4.5×10^{-9} M for paraoxon and 5×10^{-9} M for diazoxon. A comparison of these values with those given in Table IV for cholinesterase inhibition points to a preference of DDVP and paraoxon for the ali-esterase and of diazoxon for the cholinesterase. The I_{50} values for tetram and QAT were found to be much higher ($> 10^{-8}$ M), after 1 hr incubation. Because of the very low reaction rates (see Table I) much lower I_{50} values may be expected if an "infinite incubation time" would be allowed.

b. The ali-esterase activity towards methylbutyrate in AD-treated S-fly homogenates and fresh D-fly homogenates was only 10 to 20% of that in fresh S-fly homogenates. These low activities made it more difficult to obtain reliable inhibition data. Moreover it was found that the hydrolysis of methylbutyrate by these homogenates was partly due to enzymes that are qualitatively different from those acting in the S-homogenates: a considerable part (up to 50%) of the hydrolysis proved to be rather organophosphate-insensitive. The organophosphate-insensitive enzyme may also be present in the fresh S-homogenates but there it is responsible for a negligible part of the total activity (VAN ASPEREN & OPPENOORTH, 1959).

Rough estimates of the I_{50}-values, considering only the organophosphate-sensitive part of the activity, for AD-treated S homogenates were 1.5×10^{-9} for DDVP, less than 1×10^{-9} M for paraoxon and 1.5×10^{-9} M for diazoxon. The respective data for D-homogenates were 5×10^{-9} M, 2×10^{-9} M and 4×10^{-9} M. These results point to qualitative differences between AD-treated S-homogenates and D-homogenates with respect to their esterases, though the overall activities towards methylbutyrate under our experimental conditions were about equal.

3. *The presence of phosphatases or organophosphate binding compounds.*

The reaction between an organophosphate and a homogenate results not only in inhibition of the esterases but also in the disappearance of free organophosphate. Taking into account that in the experiments on whole fly homogenates (Figs. 1, 2 and 3) 1.5 flies were used in 2 ml of reaction medium it can be calculated that practically complete inhibition of the cholinesterase (and the aliesterase) is accompanied by the disappearance of the following amounts of organophosphate:

S-homogenates	D-homogenates	
14	8	μμ moles of DDVP/fly
16	16	,, ,, ,, paraoxon/fly
11	10	,, ,, ,, diazoxon/fly

These are rough estimates only, which nevertheless clearly show that the amounts of organophosphate that have to be added in order to obtain practically complete inhibition of the esterases and which are used up in this reaction, tend to be smaller in the D- than in the S-homogenates.

The data given above only refer to the amounts of organophosphate that are eliminated during the period in which the inhibition of the esterases goes to completion. Probably this is, at least partly, due to the inhibition process (phosphorylation of the enzymes). Therefore, a study was undertaken to determine the amounts that can be eliminated if larger quantities are incubated with the homogenates for longer periods of time. Since in these experiments the inhibition of the esterases in the homogenates was always complete, fresh flyhead homogenates were added at the end of the incubation period in order to check the presence and to estimate the amounts of organophosphate.

Table V gives results of experiments with DDVP, paraoxon and diazoxon. These may be briefly summarized as follows: the amounts eliminated by S-homogenates are only a little higher than those used up during the inhibitory process. This also applies to the D-homogenates in the case of DDVP. Entirely different results were obtained in the experiments on D-homogenates with paraoxon and diazoxon. The amounts eliminated are much larger than those calculated from Figs. 2 and 3. This must mean that in the D-homogenates a paraoxon- and diazoxon eliminating capacity is present that is not apparent in the short term experiments on inhibition (Figs. 2 and 3) but which shows up clearly in the long term experiments (Table V).

TABLE V

Elimination of organophosphates from whole fly homogenates

Compound	Amount ($\mu\mu$ moles/fly)				
	added	recovered	eliminated	recovered	eliminated
		in Strain S		in Strain D	
DDVP	7	—	—	0.5	6.5
	15	0.5	14.5	6	9
	20	3	17	—	—
	30	8	22	—	—
Paraoxon	15	—	—	0	15
	20	2	18	0	20
	30	10	20	—	—
	40	20	20	4	36
Diazoxon	15	0	15	—	—
	20	4	16	0	20
	30	14	16	0	30
	40	—	—	0	40

Procedure: A whole fly homogenate in 3.5% NaCl-solution was prepared containing 1 fly and the indicated amount of organophosphate per ml. This was incubated for 3 hrs at 27° C, after which 0.25—1 ml was transferred to a Warburg flask containing 0.8 ml of a freshly prepared flyhead homogenate (3 heads/ml in 3.5% NaCl). After 1.5 hrs of incubation acetylcholine (final conc. 1.5×10^{-2} M) and $NaHCO_3$ (final conc. 0.025 M) were added and the cholinesterase activity measured. By comparing this with those found in flyhead homogenates after addition of known amounts of organophosphate, the amount present in the whole fly homogenate could be calculated.

1 $\mu\mu$ mole $= 10^{-12}$ moles. This corresponds to 0.000221 μg DDVP, 0.000275 μg paraoxon and 0.000288 μg diazoxon.

Some more experiments were then performed in order to obtain information on the velocity and the nature of the reaction by which diazoxon is eliminated in D-homogenates. In one experiment 40 $\mu\mu$ moles of diazoxon were added and the amounts present after 60, 80 and 100 min. were found to be 12, 3 and 0 $\mu\mu$ moles respectively. Although these data for technical reasons are quantitatively inaccurate they strongly point to a rather surprising independence of the reaction velocity from the diazoxon concentration. This was further investigated by another experiment, in which both the homogenate and the diazoxon concentration were four times increased, i.e. 1 ml of homogenate contained 4 flies and 160 $\mu\mu$ moles of diazoxon. Complete elimination of the diazoxon added took about 70 min., which is only a little less than in the experiment referred to above. Probably, therefore, the elimination process is a zero order reaction and has an enzymatic character.

Since elimination of organophosphates was thought to be an important factor in the mechanism of resistance, another strain (C), showing a much higher level of resistance, was also studied. The capacity of degrading paraoxon and diazoxon proved to be much higher in the C-homogenates than in the D-homogenates (Table VI). Comparison of the amounts eliminated in 2 and in 4 hours and of the data obtained under quite different conditions of concentration (H_6 and H_1) again points to a marked independence of the rate of elimination from the organophosphate concentration. It seems thus reasonable to assume the presence in the D- and C-homogenates of a phosphatase, which can hydrolyse diazoxon and paraoxon, though the hydrolytic nature of the elimination reaction has not been proved.

TABLE VI

Elimination of organophosphates from whole fly homogenates (Strain C)

Whole fly homogenates containing different amounts of organophosphate were incubated at 27° C. After 2 or 4 hrs of incubation fresh fly head homogenates were added and their ChE inhibition measured in order to check whether organophosphate was still present. In this way it was possible to determine what amount of organophosphate could just be eliminated in the time available. These amounts are given in the table, expressed as $\mu\mu$ moles/fly. Two different homogenate concentrations were used: H_1 containing 1 fly/ml, and H_6 containing 6 flies/ml and correspondingly 6-fold increased amounts of organophosphates.

Organophosphate used	Homogenate conc.	Amounts eliminated ($\mu\mu$ moles/fly) 2 hr incub.	4 hr incub.
Paraoxon	H_6	310	580
,,	H_6	250	500
Diazoxon	H_6	230	470
,,	H_6	310	580
,,	H_6	232*	—
,,	H_1	235†	—

* Mean of 5 determinations † Mean of 2 determinations.

DISCUSSION AND CONCLUSIONS

The concept of competition

If the incubation time is "infinite" the reaction between an enzyme and an irreversible inhibitor is a simple titration, i.e. the number of inhibitor molecules required to obtain 100% inhibition will be equal to the number of enzyme molecules (or active sites) present. If a plot is made of the percentage inhibition versus the inhibitor concentration a straight line will result. A more complicated situation is likely to be found if the behaviour of an inhibitor is studied in crude homogenates. Other compounds also reacting with the inhibitor will often be present and interfere with the simple relationship described above.

The purpose of the present study is to describe and to explain the relationships observed if organophosphates are added to crude homogenates of house fly tissues. In these crude homogenates several components reacting with organophosphates were found to be present. They may be listed as follows: 1. a cholinesterase (splitting acetylcholine) 2. an ali-esterase (splitting methyl- and phenylbutyrate) and 3. a phosphatase (splitting diazoxon and also paraoxon). Other compounds, probably proteins, reacting with organophosphates are likely to be present. The constituents 1 and 2 react with organophosphates in a simple mole to mole reaction, resulting in both inactivation of the enzymes and elimination of the organophosphates. The phosphatase acts as a catalyst in the elimination of one or two of the organophosphates. Though the picture may be incomplete it is thought to be suitable for explaining the observed facts on the basis of the concept of competition given in the introduction.

There is little doubt that the hydrolysis of substrates and the reaction with organophosphates occur at the same site of the ali-esterase molecule. AD-treatment, which interferes with the hydrolytic activity, will therefore also interfere with the organophosphate binding capacity of this enzyme and consequently remove one of the competitors for the organophosphate. Accordingly AD-treatment was found to increase the inhibition of the cholinesterase (Tables II, III and IV; Figs. 1, 2 and 3) by DDVP, paraoxon and diazoxon. It did not affect the inhibition by Tetram and QAT. This would be expected since the rate constants given in Table I clearly show that these two compounds strongly prefer to react with the cholinesterase, so that the ali-esterase will hardly be able to compete.

Organophosphate resistant flies contain much less ali-esterase than susceptible flies. This is clearly reflected in the much stronger inhibition of the cholinesterase in D- than in S-homogenates as found in the experiments in which DDVP and paraoxon were used (Figs. 1 and 2, Table IV). The difference was much smaller or absent in the experiments on diazoxon inhibition (Fig. 3, Table IV). As anticipated, AD-treatment of D-homogenates generally did not strongly affect the cholinesterase inhibition and resulted in curves that are about similar to those obtained with AD-treated S-homogenates. The shift was somewhat more distinct in the experiments with diazoxon (Figs. 1, 2 and 3).

So far no mention has been made of other compounds involved in the competition. Their presence might be reflected in the deviation from linearity in the lower parts of the curves obtained with AD-treated homogenates and paraoxon or diazoxon (Figs. 2 and 3). If such compounds are actually present they must have high affinity for paraoxon and diazoxon and low affinity for DDVP, since with the latter compound no competitive effect is observed (Fig. 1). All this is rather hypothetical.

The phosphatase found to be present in the D-homogenates (Table V) might be expected to compete strongly with the cholinesterase, at least for paraoxon and diazoxon. Figs. 1, 2 and 3 clearly show that its presence does not compensate for the loss of ali-esterase, except probably in the experiments on diazoxon. This would mean that it is a less active competitor than the ali-esterase for DDVP and paraoxon and possibly about equally active as the ali-esterase in competing for diazoxon.

The mechanism for resistance

The strains D and C have been shown to differ in three respects from the susceptible strain: they are resistant to some organophosphorus insecticides, they have low ali-esterase activity and they contain a phosphatase which degrades the organophosphates. Since Nguy & Busvine (1960) found that the resistance in both strains is due to a single gene, the question arises as to whether the two other differences are also dependent on this gene. It has been shown that in two other resistant strains low ali-esterase activity depends on the presence of one gene which is also responsible for resistance (OPPENOORTH, 1959). Therefore, it is very likely that the one gene for resistance in strain D and C is identical with that causing low ali-esterase activity in these strains. The fact that the phosphatase in strain D can degrade paraoxon and diazoxon, to which the strain is resistant, but cannot degrade DDVP, to which it is susceptible, indicates that the phosphatase is indeed responsible for the resistance. Moreover, the activity of the phosphatase in strain C is much higher than in strain D, which closely corresponds with the level of resistance found in these strains. From these findings it can be concluded that the three differences all depend on the same gene, and that the presence of the phosphatase constitutes the only resistance mechanism.

It seems justified to consider the phosphatase as a modification of the ali-esterase present in susceptible flies, due to mutation of a single gene. This mutation at the same time caused the loss of hydrolytic activity to esters such as methylbutyrate and conferred the ability to split paraoxon and diazoxon.

The experiments reported in the present paper indicate that in homogenates the absence of the ali-esterase will cause an apparently increased sensitivity of the cholinesterase and of any other receptor molecule, at least if it has not a much greater affinity for the organophosphates than the ali-esterase. The absence of ali-esterase in D- and C- flies would therefore be expected to increase their susceptibility to organophosphates. This seems to be in striking contrast with the observation that D- and C- flies show considerable resistance to paraoxon and diazoxon due to the genetical factor for low ali-esterase activity.

However, D- and C-homogenates do not only differ from S-homogenates by their lower ali-esterase content, they also contain a phosphatase, which is absent from S-homogenates. The loss of ali-esterase might thus be compensated or even more than that by the appearance of this phosphatase. It may be objected that the experiments on competition in homogenates reveal that the phosphatase in D-flies generally shows less competitive potency than the ali-esterase in S- flies. Only in the case of diazoxon the potencies of phosphatase and ali-esterase were found to be about equal. However, a living fly does not behave like a homogenate: structural barriers are likely to be present which change the "free competition" such as occurs in a homogenate, into a more "directed economy". This change could bring the phosphatase into a much better position for competition, so that its ability to eliminate theoretically unlimited amounts of paraoxon and diazoxon would show to full advantage. Interference of structure with free competition was recently revealed by a study on *in vivo* inhibition of the cholinesterase and the ali-esterase in houseflies (VAN ASPEREN 1959). The inhibition of the ali-esterase was found to occur much more rapidly than the inhibition of the cholinesterase and the difference was much greater than would have been expected in the case of free competition.

The activity of the phosphatase in our experiments proved to be nearly independent of the concentration of its substrate, diazoxon. Though the concentration of diazoxon in a fly treated with this compound in general will be much higher than that present in our *in vitro* experiments, the rate of elimination cannot be expected to be appreciably higher than that found in the homogenates, which amounted to approximately 34 $\mu\mu$ moles/hr/fly or 0.010 μg/hr/fly in strain D and 120 $\mu\mu$ moles/hr/fly or 0.036 μg/hr/fly in strain C. This rate of elimination is surprisingly low as compared to the LD_{50} values (topical application in acetone, which results in rapid absorption) which are in the order of 0.03, 0.07 and 0.5 μg/fly in strains S, D and C respectively. Probably, therefore, the phosphatase is unable to reduce the total amount of organophosphate rapidly enough to a harmless level. Yet, there can be no doubt that it plays an important part in the defence mechanism of the resistant flies. It seems likely, that its function is to prevent fatal concentrations from building up near the site of toxic action. It will be clear that it can only accomplish this task in co-operation with structural factors causing an uneven distribution of the inhibitor in the fly body. Another possibility would be that the conditions in the living fly are such that much higher elimination rates than those reported above are obtained. The latter possibility cannot be excluded, since MENGLE & O'BRIEN (1959) have shown the presence in houseflies of a component that favours dephosphorylation of the phosphorylated cholinesterase. As dephosphorylation of the phosphatase could well be the rate limiting step in the enzymatic reaction, the occurrence of such components might increase the turnover rate of the phosphatase as well.

Biochemical implications

Since the phosphatase has to be considered as a modification of the ali-esterase present in S-flies, due to mutation of a single gene, it is highly probable that the shift of the concentration-inhibition curves (Figs. 1, 2 and 3) is due to the disappearance of the ali-esterase only. If so, the concentration of the ali-esterase in the S-homogenates must be equal to or larger than the concentration difference covered by the observed shift. This difference is about 5 $\mu\mu$ moles/ml in the experiments on DDVP and paraoxon (Fig. 1 and 2), from which it can be calculated that the amount of ali-esterase would be at least 7 $\mu\mu$ moles per fly. From the I_{50}-values found for the ali-esterase in S-homogenates it can be deduced that the amount of ali-esterase can maximally amount to 12 $\mu\mu$ moles per fly. It can further be calculated that the ali-esterase of one fly, at full saturation with its substrate, can hydrolyse approximately 700.000 $\mu\mu$ moles of methylbutyrate per min. under the experimental conditions described. Most probably, therefore, the turnover number of the ali-esterase will be in the range from 60.000 to 100.000 per minute.

Assuming that the amount of phosphatase in C- and D-flies is equal to the amount of ali-esterase in an S-fly the turnover number of the phosphatase against diazoxon would be in the order of 0.05—0.08 per min. in strain D, and in the order of 0.17—0.29 per min. for strain C.

The assumption just made is quite plausible on theoretical grounds but certainly needs experimental confirmation.

Some remarks may finally be made with respect to the use of the term "phosphatase" in the present paper. The evidence that the enzyme degrading organophosphates is a modification of the ali-esterase present in susceptible flies strongly favours the view that the degradation is a hydrolytic process (see Introduction). Since, moreover, organophosphates are very labile to hydrolysis, it seems justifiable to use the term "phosphatase", which may be defined as "an enzyme hydrolysing ester of orthophosphoric acid". As long as nothing is known about its substrate specificity and possible physiological functions, this name should be considered a preliminary one.

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ZUSAMMENFASSUNG

DIE WECHSELWIRKUNG ZWISCHEN ORGANISCHEN PHOSPHOR-INSEKTIZIDEN UND ESTERASEN IN HOMOGENISATEN VON PHOSPHORESTER-EMPFINDLICHEN UND -RESISTENTEN STUBENFLIEGEN

Wenn einem Stubenfliegen-Homogenisat Phosphorsäure-Ester zugesetzt werden, dann werden mehrere Esterasen in wechselseitiger Konkurrenz mit diesen Substanzen zu reagieren versuchen. Die wichtigsten sind die Cholinesterase und eine Aliesterase, die zur Hydrolyse des Methyl- und Phenylbutyrats und mehrerer anderen Ester befähigt ist. Nach Zusatz geringer Mengen von Phosphorsäure-Ester wird das Endergebnis dieses Wettbewerbes von den Quantitäten der Esterasen und ihren Reaktionsgeschwindigkeiten abhängig sein.

Tabelle I zeigt die bimolekularen Geschwindigkeitskonstanten für die Reaktionen zwischen der Cholinesterase und der Aliesterase und den fünf untersuchten Estern: DDVP, Paraoxon, Diazoxon, Tetram und QAT (die quaternären Phosphonothiolat-Analoge von Tetram). Aus diesen Daten wird geschlossen, daß die Aliesterase ein wichtiger Konkurrent der Cholinesterase für DDVP, Paraoxon und Diazoxon darstellt. Dementsprechend wurde gefunden, daß ach Beseitigung der Aliesterase mittels einer Denaturierung (AD-Verfahren) die Hemmung der Cholinesterase durch diese drei Substanzen bedeutend höher ist (Tabelle II, III und IV; Fig. 1, 2 und 3), nicht aber jene durch Tetram und QAT.

In Homogenisaten von Phosphorester-resistenten Fliegen, die — wie früher gezeigt wurde — viel weniger Aliesterase enthalten, war die Hemmung der Cholinesterase meistens viel stärker als in „empfindlichen Homogenisaten" (Tabelle IV; Fig. 1, 2 und 3). Es erschien schwer, diese Befunde mit dem früheren in Einklang zu bringen, daß niedrige Aliesterase-Aktivität und Resistenz eng verbunden seien. Weitere Versuche aber zeigten, daß resistente Fliegen eine modifizierte Aliesterase enthalten, die keine oder eine nur geringe Aktivität gegenüber Methyl- und Phenylbutyrat aufweist, aber die Fähigkeit besitzt, die Phosphorsäure-Ester, gegen welche Resistenz besteht, abzubauen. Die Substrat-Spezifizität und Aktivität wiesen eine deutliche Korrelation mit der Art und Höhe der Resistenz auf.

Die Umwandlung der Aliesterase (in empfindlichen Fliegen) in eine „Phosphatase" (das modifizierte Enzym in resistenten Fliegen) wird durch eine einzige Gen-Mutation verursacht. Diese ist in den zwei untersuchten Stämmen auch für die Resistenz verantwortlich.

Die Phosphatasen haben eine hohe Affinität für ihre Substrate, Paraoxon und Diazoxon. Die Abbau-Geschwindigkeit in vitro (0.01—0.04 μg/St/Fliege) ist aber niedrig im Vergleich mit den gefundenen LD_{50}-Werten. Da ihre Bedeutung für den Mechanismus der Resistenz feststeht, muß entweder angenommen werden, daß der Abbau in vivo schneller verläuft oder daß strukturelle Faktoren in der lebenden Fliege einen wichtigen Einfluß ausüben.

Weitere Betrachtungen ermöglichten die Berechnung der wahrscheinlichen Aliesterase- und Phosphatase-Konzentration und der Umsatzzahlen für Diazoxon in Homogenisaten.

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OBSERVATIONS ON THE OVIPOSITION BEHAVIOUR OF FRIT FLY *(OSCINELLA FRIT* L. DIPT. CHLOROPIDAE)

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Two separate groups of stimuli (1) originating from the plant, (2) proprioceptive, have been found to mediate egg laying. An artificial shoot has been devised to investigate oviposition behaviour.

In England, frit fly usually has three generations each year. Adults of the first generation appear in May and lay eggs on young oats. The second generation lays on the developing spikelets in summer. The third generation lays on grasses in autumn (Cunliffe 1921, 1924; Jepson & Southwood 1958). However, all three generations may also be found on grasses.

As oviposition sites are so diverse, there has been little attempt to find common factors which may mediate oviposition. Attention has more usually been directed to differences in susceptibility between varieties of oat, differences which are, however, themselves apparently due to preferential oviposition (Cunliffe & Hodges 1946; Bingham & Lupton 1958). It has been found that the emergence of frit fly is commonly considerably delayed in the N.E. in Northumberland, owing to the colder Springs (Ibbotson in prep.) so that egg laying coincides with later stages in the development of the normal oat crop than it does further south. The first generation will, apparently, lay eggs either on developing spikelets or on tillers at the base of the plant, according to their respective availability. Factors mediating oviposition may to some extent be common to all sites and an investigation was made with a view to their preliminary analysis. In particular, it seemed desirable to find an artificial shoot substitute, which the female flies would accept for oviposition as readily as a live shoot, in order that, in subsequent work, individual characteristics could be altered without destroying the rest by killing the shoot.

METHODS

The experiments and cultures were kept in an insulated room maintained at about 24° C. Quantities of damaged tillers were taken from the field in July, confined to a small insectary and the flies collected as they emerged. They were separated in batches of twenty, each beneath a hurricane lamp glass capped with cotton fabric and standing on a block of plaster of paris in a tray of water. With this arrangement, the humidity inside the glasses (as measured by an Edney paper hygrometer) remained at about 75% R.H. even though the humidity in the room itself was usually about 45% R.H. or less. The cultures were all illuminated from above by a 60 watt electric light bulb attached to a time clock adjusted for a 16 hr. day and the flies were periodically fed through the cotton on honey and water.

Most of the trials involved the use of standard live shoots for comparison. These were obtained by planting oat grains (var. Blenda) in compost, 24 to a 15 cm flower pot, so that the upper end of the grain was just visible at soil level. When germination had begun the pots were covered with a further thin layer of soil and kept in the open air (beneath wire netting to protect them from sparrows). Single bladed shoots, between 5 and 7.5 cm long, were removed from the pots as required.

All forms of shoot or shoot substitute used, were contained individually in 2.5 × 2.5 cm flat bottomed, glass specimen tubes, two of which just fitted into one lamp glass. The numbers of eggs laid on a variety of model shoots, were compared with the numbers laid on the appropriate controls, by confining model and control beneath a lamp glass for 2 or 3 days and counting the eggs laid on each at the end of the period. In all, twenty culture glasses were maintained and successive comparisons were assigned to them at random.

RESULTS

From time to time, the behaviour of the flies was carefully watched over a considerable period, both in the lamp glasses and, beneath a binocular microscope, in a smaller tube to which apparently fecund females and a live shoot were periodically confined. In both types of container, the flies spent the majority of the time either at rest or walking about on the underside of the cotton ceiling, with occasional short flights and one or two were always to be seen, sitting in the typical head down position on the shoots themselves. Individual flies often made irregular circus movements on the base of the shoot itself and out on to the surface round it. These either protracted the proboscis and sampled the substrate from time to time or attempted to oviposit, protracting the ovipositor and thrusting it into crevices or over the side of declivities in the soil. The flies were distinguished from each other, partly by proboscis and ovipositor which were just visible to the naked eye and partly by the movements of flies which were ovipositing, which were considerably the more jerky and hurried.

Ovipositing flies frequently halted, extended the telescopic ovipositor and depressed the end of the abdomen, with fidgeting, downward thrusting movements. The fly became less restless after the ovipositor had been fully extended and as seen beneath the microscope, remained more or less motionless, with the tip of the ovipositor writhing back and forth and from side to side in a prehensile manner, as though seeking a particular pattern of tactile stimulation. Movements such as these were frequently abortive and the fly would then withdraw the ovipositor partly into itself, run a short distance in an irregular manner and repeat the process.

Occasionally, however, the fly became completely stationary and after a short period, three or four contractions of the abdomen would slowly expel an egg. When viewed in profile at this time, under magnification the ovipositor was seen to be appressed at the tip to the substrate and when the egg emerged, although not deformed in shape, it appeared to be pressed lightly against the surface on which it was being laid, at the same time as the ovipositor was withdrawn. When oviposition was completed, or, sometimes after an abortive attempt, the fly ran back up the shoot, reversed its position and after a period during which it made cleaning motions with the fore tarsi, remained in the head down position for some time.

The flies were never seen to lay more than one at a time, but eggs may sometimes have been laid together, as they often appeared in close aggregates on the surface of the soil. Ovipositing flies did not appear to feed at all, the proboscis remaining retracted throughout the entire series of movements.

In a number of trials the base of the shoots was invested and the soil covered, with smooth paraffin wax or cotton wool, or the soil was replaced by coarse sand. The numbers of eggs laid on these treatments and on the controls with which they were paired, are given in Table I Series 1.

TABLE I

Eggs laid per replicate by O. frit *in choice experiments, in which oat shoots in soil were compared with oat shoots invested round the base by other materials, as potential sites for oviposition*

Series 1. Shoots in soil paired with other treatments beneath lamp glasses

Treatments	Number of eggs														
Soil	6	10	1	12	8	8	2	16	1	5	2	1	6	10	9
Coarse sand	5	3	10	9	14										
Smooth wax						0	0	0	1*	0					
Cotton wool											0	0	0	2*	1*

* These eggs were all laid on the shoot itself.

Series 2. (In these trials, three separate shoots were contained together in a large glass vessel)

Shoots in soil	10	24	12
Soil alone	0	0	0
Shoot in split cork	0	0	0

Notes

Shoot in split cork: soil surface covered with a layer of smooth cork bored to fit the shoot and then split in half to fit round inside the top of the specimen tube containing the shoot.

Shoot in wax: as above, with paraffin wax painted over the cork surface and the base of the shoot.

Shoot on cotton wool: the tube half filled with soil only, the rest with cotton wool.

Fewer eggs were laid where the soil was covered with cotton wool or wax and as many where it was replaced with sand, as were laid on the controls. In all, eight actual depositions were observed beneath the microscope, two on the shoot itself, one against the glass of the containing tube, one on sand and four on soil, together with a large number of abortive attempts, principally on wax and cotton wool. Attemps to lay eggs on wax failed owing apparently to the difficulty of extending the ovipositor to the full on a flat surface (Fig. 1).

When the stance was normal, the fully protracted ovipositor extended beyond and considerably below the level of the hind tarsi and the flies made continual attempts to find cracks or declivities, which would enable them to adopt the correct posture. On the flat surface, the ovipositor could only be extended with an upward bend, which presumably prevented it from obtaining the appropriate

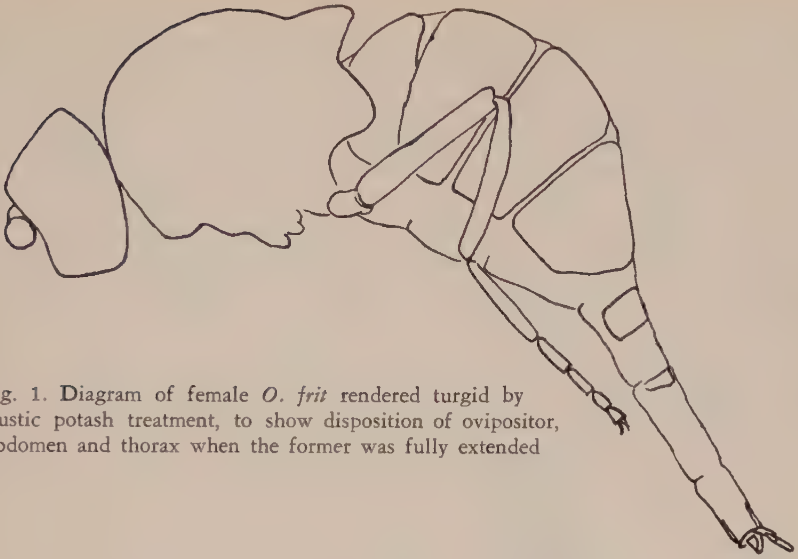

Fig. 1. Diagram of female *O. frit* rendered turgid by caustic potash treatment, to show disposition of ovipositor, abdomen and thorax when the former was fully extended

contacts. On cotton wool, on the other hand, although fully extended down into the fibres, the tip of the ovipositor writhed continually back and forth and from side to side, as though seeking a steady surface. At the same time the fly made repeated, hurried readjustments of posture, apparently in a attempt to obtain firm contact with the tip. The absence of this appeared to act, equally with the absence of good contacts on the flat surface, in completely inhibiting the expulsion of eggs. Flies which had been attempting to oviposit on wax or cotton wool for some time, were occasionally transferred to a tube containing a live shoot in coarse sand or soil and laid eggs almost immediately. Tactile stimulation of both dorsal and ventral surfaces of the ovipositor together during deposition appeared to be unnecessary.

Under high magnification, the ovipositor was seen to be furnished along its length with groups of what were apparently sensory bristles, contact with one or more of which may have been a necessary precursor to egg laying.

As no eggs were laid on the soil in the absence of shoots (Table I Series 2.), the soil had no special value of its own in mediating oviposition apart from its tactile qualities. Eggs may have been absent from the cork surface, either because it did not afford sufficient inequalities for the reception of the ovipositor or because it was completely dry, but no further trials were made at this point.

The actual distribution of eggs on the surface of the soil round separate shoots and the frequency distribution in mm of the distance between individual eggs and shoots in the first ten replicates to contain eggs is given in Figs 2a and 2b.

A smooth round shoot appeared to afford few opportunities for the females to obtain the appropriate physical contacts with the ovipositor and eggs laid on the soil seemed to be deposited by flies which moved away from the original shoot in their attempts to find suitable tactile co-ordinates. The eggs laid on the shoots themselves were deposited beneath the coleoptile or by flies which straddled the

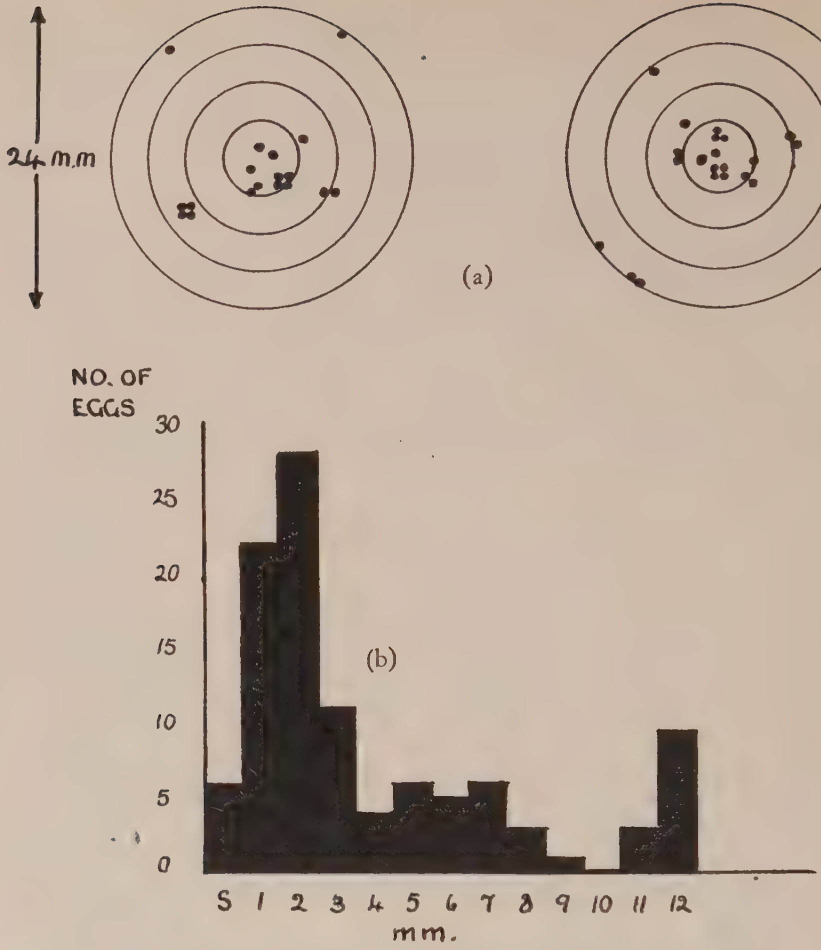

Fig. 2. (a) Distribution of eggs laid by *O. frit* on soil surface round oat shoots (b) Frequency distribution of distance in mm between eggs laid by *O. frit* and oat shoots in ten replicates containing eggs

gap between shoot and soil. The former were usually laid by flies sitting diagonally, in such a manner that the ovipositor extended round the curve of the stem. It is interesting to note that BELYAEV & KARCHENKO (1936) working on the factors which determine resistance in wheat, found that the young stems of resistant plants were coarse (i.e. thick?) by comparison with those of plants which were susceptible, suggesting that on thicker shoots there were fewer opportunities still for the flies to adopt the necessary posture.

Nevertheless, oviposition did not occur with the provision of suitable tactile co-ordinates alone. Flies confined over waxed rods in soil, i.e. in a suitable environment but for the presence of the host plant, failed to lay eggs over a period of six weeks, although the females were found to contain eggs when periodically dissected and laid eggs readily when individuals were provided with suitable hosts in soil in separate containers.

It seemed possible to consider the total effective stimulus pattern as consisting of two parts in series viz; first, a (probably biochemical) component originating from the plant itself and second, a proprioceptive component resulting from the disposition of legs and ovipositor, which was not necessarily dependant on the presence of the host at all.

A preliminary analysis of the former was effected by comparing, in the same manner as before, the numbers of eggs laid on a variety of models with the numbers laid on or around live shoots or the appropriate controls. Apart from the standard shoot finally developed, these models were all associated with suitable tactile co-ordinates, as they were all in soil. The distinction between proprioceptive and host-originated stimuli was confirmed by the fact that no continually abortive attempts at oviposition, such as occurred with cotton wool or wax were ever seen. The results are given in Table II, together with notes on the construction of the various models.

TABLE II

Eggs laid per replicate by O. frit *in choice experiments in which oat shoots or other controls were compared with model shoots, as potential sites for oviposition. All in soil.*

Treatment	Number of eggs														
Series 1															
Live shoots	17	6	10	8	12	6	17	8	8	2	2	2	9	4	10
Waxed shoots	2	0	1	1	0										
Rolled filter paper + oat extract						0	0	0	0	0					
Filter paper, waxed rod + oat extract											8	0	4	11	12
Series 2															
Filter paper, waxed rod + oat extract	11	6	4	15	9	6	8	5	3	10					
-do- less oat	0	0	0	0	0										
-do- less wax						6	14	2	3	15					
Series 3															
Oat shoot in soil	4	22	0	9	10	8	3								
Standard artificial shoot	7	8	9	10	6	4	11								
Series 4															
Live shoots	24	6	12	4	9	2	20	12	10	9					
Green oat straw	1	0	2	0	3										
Wilted oat shoot in soil						1	6	2	0	1					

Notes

Waxed shoot: shoot painted completely with low m.p. paraffin wax (38° C), before planting. This wax stretched with the growth of the shoot and continued to cover it completely for 48 hrs.

Rolled filter paper: see Fig. 3 (b); initially, (Series 1 above), it was also rolled on to a glass rod 1½ mm in diameter covered with wax, so that it stuck, leaving a half open channel down one side.

Standard artificial shoot: since wax was not essential for oviposition (Series 2 above) a simplified artificial shoot was constructed by applying a triangle of filter paper moistened with oat extract to the inside of a 2.5 × 2.5 cm specimen tube where it was held by surface tension.

Eggs were all deposited around the edge, between the filter paper and the glass.

All the eggs were laid on the artificial shoots between filter paper and core and not, as they were with live shoots, in the soil. To the unaided eye the behaviour of the flies suggested that the smooth round live shoots themselves simply provided few situations with the appropriate tactile co-ordinates by comparison with the models. If this was correct, then the facts that eggs were only laid occasionally on the waxed shoots and not at all in the absence of oat extract, confirmed that at least a part of the host-originated stimulus pattern was provided by the tissue itself. Moreover, the absence of eggs from some of the models treated with oat extract pointed to the possible existence of an additional factor. This was embodied in the difference between treated filter paper investing a filter paper core and treated filter paper investing a waxed or glass rod and could have consisted of the proximity of a smooth or neutral surface. However, as the immediate object of the work was to construct a standard artificial shoot for test purposes, further analysis was postponed at this point. The two provisional components were embodied in a model, which the flies were found to accept for oviposition as readily as a live shoot (Table II Series 3). There was no evidence in these experiments that the flies discriminated visually between sites as the model bore little resemblance to a living plant.

A few eggs were also laid in the vicinity of, or on, both green oat straw and oat shoots which had wilted and were almost dead (Table II Series 4). This suggested that gradations were possible in the strength of the host-originated stimuli and that young growing tissue was their best source. If these results are substantiated by further work it is, of course, along such lines as these, that explanations may be sought for real differences in suitability for oviposition between plants in the field.

The results of some preliminary trials using the standard artificial shoot, in which, chiefly, extracts from other possible hosts were used instead of oat extract, are given in Table III.

TABLE III

Eggs laid by O. frit *on standard artificial shoots moistened with extracts of oat shoots and other possible hosts, or sugar*

Treatment	Number of eggs laid															
Oat extract	7	4	11	5	6	3	3	8	1	3	8	8	9	3	4	9
Water	0	0	0	0												
Sugar (Sucrose)					0	4	0	0								
Wheat shoot extract									10	0	0	1				
Barley shoot extract													1	4	0	0

By this time, egg laying in the cultures had begun to diminish, so that the figures do little more than suggest possible trends. So far as they go, however, in view of the fact that no eggs were laid at all when the filter paper was moistened with water, they suggest that wheat and barley extracts were not entirely ineffective. On the other hand, a few eggs were deposited on the sugar treatment but none where the filter paper, previously moistened with oat extract, had been allowed to dry out. The stimuli which mediate feeding may, clearly, be complementary to those which mediate oviposition, the only evidence of any difference between them being the fact that flies were not seen to protract their probosces at all during oviposition. This may well have occurred, however, before ovipositing flies could be specifically identified.

Although set in a somewhat different context from the one in which Cunliffe worked, the above results tend to confirm his general opinion, viz; "It would seem, then, that two factors were operating to determine the extent of oviposition, namely (1) plant attractiveness......... (2) coleoptile attractiveness, a definite stereotactic factor resulting in increased deposition on plants with open coleoptiles and change in the coleoptile/soil ratio" (of eggs laid).

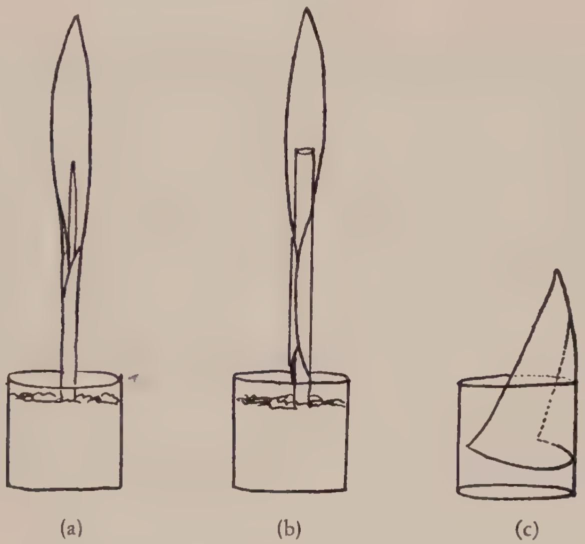

Fig. 3. Diagrams of three principle oviposition targets (a) Live oat shoot in soil (b) Glass rod 1½ mm in diameter invested by filter paper (c) Final, artificial shoot

A number of authors have mentioned that the degree of separation of the coleoptile from the stem affects the amount of oviposition and it seems unnecessary to postulate any more complex a situation, than the relatively simple provision of suitable tactile co-ordinates for the act of oviposition itself. Eggs found in the soil in the field, may well be deposited by flies that have been carried out on to the soil in their attempts to arrive at a suitable posture and the area over which they are deposited may well be larger than in the laboratory, owing to the greater light intensity and greater amount of activity of the flies.

On the other hand, the composition of the stimulus pattern provided by the shoot itself seems more complex. That there should be some factor derived from the shoot itself which is embodied in shoot extract, is perhaps to be expected. That this must be associated with the proximity of a particular surface seems unusual and how this association facilitates host and site selection in the field, is as yet difficult to understand. The only evidence apart from the above of the existence of such a factor is provided by Belyaev & Karchenko (1936) who, working on the factors which affect resistance to frit attack in wheat, found that the leaf sheaths of resistant varieties were clearly ribbed, whereas those of susceptible varieties were smooth.

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ZUSAMMENFASSUNG

BEOBACHTUNGEN ÜBER DAS EIABLAGEVERHALTEN DER FRITFLIEGE (OSCINELLA FRIT *L. DIPT. CHLOROPIDAE*).

Die Eiablage wird durch eine Anzahl von Reizen ausgelöst, die nacheinander wirken, jedoch im einzelnen nicht genauer identifiziert wurden. Sie lassen sich jedoch in zwei Gruppen trennen: (1) Reize, die von der Wirtspflanze selbst ausgehen und (2) solche, die wahrscheinlich propriorezeptiv wirken und auf der Notwendigkeit einer geeigneten Haltung der Beine und des Legeapparates während des Eiablageaktes beruhen.

Die propriorezeptiven Reize scheinen die Fliegen häufig von den Keimpflanzen weg und zur Ablage ihrer Eier auf dem Boden zu führen, was darauf hindeutet, daß die Eier im Freien in ähnlicher Weise in den Erdboden abgelegt werden.

Ein Extrakt aus in Wasser zerriebenen Haferpflanzen scheint in Verbindung mit einer geeigneten Oberfläche (z.B. Erdboden oder Sand) Eiablageverhalten auszulösen. Verschiedene Resultate wurden mit glatt oder rauh strukturierten Oberflächen und in Abhängigkeit von Gegenwart oder Abwesenheit des Extraktes erhalten. Die Reaktion der Fliegen scheint mit dem physiologischen Zustand des Gewebes zu variieren, das den Fliegen geboten wird, und das Eiablageverhalten ist schwach, wenn den Fliegen alte oder absterbende Pflanzenteile geboten werden. Die Wahrscheinlichkeit, daß Reize, welche Nahrungsaufnahme, und solche, die Eiablage hervorrufen, ursprünglich nicht die gleichen sind, ist nur gering.

Eine künstliche Keimpflanze, die von den Fliegen zur Eiablage ebenso angenommen wurde wie eine junge, gesunde, lebende, wurde dadurch hergestellt, daß ein dreieckiges Stück Fließpapier mit Haferextrakt befeuchtet und in das Innere einer 2,5 × 2,5 cm Glastube gesteckt wurde.

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SOME FACTORS THAT AFFECT THE ANNUAL CYCLE OF THE WINTER MOTH, *OPEROPHTERA BRUMATA* (L.) (LEPIDOPTERA: GEOMETRIDAE) IN WESTERN EUROPE

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Egg development rate in the winter moth (*Operophtera brumata* (L.)) is increased by higher temperatures between at least 6.0° C and 18° C. Pupal development rate is, in general, reduced by higher temperatures. Therefore, lower temperatures in northern localities and at high altitudes in Europe increase the duration of the egg stage and decrease that of the pupa. Inherent differences in the eggs and pupae from different localities increase the effect of temperature differences.

The winter moth, *Operophtera brumata* (L.), occurs from northern Scandinavia (KOZHANCHIKOV, 1950; NORDSTROM, 1943; SAALAS, 1949) to the south of Italy (SILVESTRI, 1941) in a variety of habitats from sea level to the deciduous tree-line. There is one generation each year: adults emerge and oviposit before the beginning of winter, and larvae hatch when buds are swelling and opening on the host trees, and pupate early in summer. Because of seasonal and phenological differences between localities, oviposition and larval hatch start in late September and early May, respectively, in Estonia (ZOLK, 1933), in November and early April in northern Switzerland (REIFF, 1953), and in December and early March in southern Italy (SILVESTRI, 1941). Therefore, and because duration of the larval stage is about six weeks (FAES, STAEHELIN & BRUDERLEIN, 1924; SILVESTRI, 1941), pupation begins in mid-April in southern Italy and mid-June in northern Europe. As a result of these differences between localities in times of oviposition, hatching, and pupation, the duration of the egg stage is only two months in southern Italy but nearly eight months in northern Europe whereas that of the pupal stage is over eight in the former region and less than three months in the latter. In addition there are differences in durations of both stages between adjacent localities at different altitudes that are correlated with climatic and phenological differences between the localities (SCHNEIDER-ORELLI, 1916; SILVESTRI, 1941). Published opinions differ as to the extents to which variations in the life cycle result from direct climatic and from intrinsic differences between populations. Several aspects of the matter are clarified in this paper by data obtained while rearing winter moth collected in western Europe (for details of the collection localities see WYLIE, 1960a). The insects were collected as larvae, reared, and shipped to Belleville soon after pupation.

ENVIRONMENTAL FACTORS

Temperature

Effects on the eggs. Mean temperature during the incubation period varies between localities in western Europe. That temperature affects the incubation period, and therefore that temperature differences are at least partly responsible for differences in the incubation period between localities, is indicated by the data in Table I for lots of 125 eggs each from Versailles, France, and Oldenburg, Germany.

TABLE I

Effect of temperature and of intrinsic differences on duration of the egg stage

Temperature	Minimum duration of egg stage (days) Versailles	Oldenburg
18° C continuously	42	47
15° C continuously	44	58
6.5° C continuously	115	129
1.5° C for 112 days; then 6.5° C	155	165
— 4.5° C for 28 days; then 6.5° C	124	152
— 4.5° C for 56 days; then 6.5° C	143	152
— 4.5° C for 84 days; then 6.5° C	165	178
— 4.5° C for 112 days; then 6.5° C	192	196
—12° C for 28 days; then 6.5° C	134	160
—12° C for 56 days; than 6.5° C	163	174
—12° C for 84 days; then 6.5° C	185	226
—12° C for 112 days; then 6.5° C	216	241
—16° C for 28 days; then 6.5° C	136	156
—16° C for 56 days; then 6.5° C	165	185
—16° C for 84 days; then 6.5° C	194	220
—16° C for 112 days; then 6.5° C	No hatching	249

Egg colour changes from green through yellow and orange to gray. The temperature threshold for development is lowest early in the egg stage: at 1.5° C freshly-laid eggs from Versailles and Oldenburg became gray in approximately eight months, but no larvae hatched; at —4.5° C they became yellow in five months, but did not develop further; and at —12° C they remained green. The minimum temperature at which freshly-laid eggs developed is, therefore, between —4.5° C and —12° C in both localities and is lower than with eggs from Leningrad, U.S.S.R. which, according to KOZHANCHIKOV (1950), do not develop in autumn below —2.5° C. This is surprising, because development at Leningrad would be expected at temperatures as low as those that inhibit it at Oldenburg and Versailles. Tests showed that the minimum temperature for larval hatch is between 1.5° C and 6.5° C for both of the latter localities; this is in agreement with the conclusion of KOZHANCHIKOV (1950) that no larvae at Leningrad hatch below approximately 6.0° C. Egg diapause occurs in autumn in the northern U.S.S.R. and is broken only by temperatures below 0° C (KOZHANCHIKOV, 1950). It is not, however, obligatory in eggs from Versailles, which develop continuously at several temperatures between 8° C and 20° C (GAUMONT, 1950). The data in Table I and similar data for eggs from Frankfurt, Germany, and from Oland and Kullaberg, Sweden, indicate that development was completed in all cases without interim exposure to temperatures below 0° C, and suggests that there is not a true diapause in these localities. The reason for egg diapause in the northern U.S.S.R. is unknown.

Effects on the pupae. The effect of temperature on pupal development during summer and early autumn was demonstrated with two lots of 800 cocoons each from Versailles. These were reared at 18° C and 15° C and a relative humidity of 80—85 per cent from August 7 until October 25, and thereafter at 6.5° C. The numbers of females that emerged were 242 and 240; the emergence ranges for the females were November 20—December 23 and November 11—December 18; and the mean emergence dates, that is, the dates on which 50 per cent of the females in each lot had emerged, were December 7 and November 29. The data indicate that more development occurred, from August 7 until October 25, at 15° C than at 18° C. This supports the conclusion of KOZHANCHIKOV (1950) that temperature inversely affects pupal development, and is contrary to that of JANCKE (1937), SPEYER (1938a), and GRISON & SILVESTRE DE SACY (1948) that temperature has no effect.

The optimum temperature for rapid development decreases as the pupae mature. At 18° C pupae developed more during the period August 7—October 25 (see emergence data above) than at 6.5° C; at the latter temperature, the emergence range for females was December 11—January 3, and the mean emergence date December 16. However, 6.5° C is more favourable than 18° C for development late in the pupal stage: this is shown by the emergence (Table II) from 300 cocoons from each of Oldenburg, Oland and Versailles previously held at 18° C and reared at 6.5° C after September 25, from three similar lots at the latter temperature after October 25, and from three after November 25.

TABLE II

Effect of high temperature late in the pupal stage on the emergence of winter moth females

Locality	Date temperature reduced from 18° C to 6.5° C	Number of females that emerged	Dates of female emergence	
			Range	Mean
Oland	Sept. 25	55	Nov. 1—Nov. 26	Nov. 13
	Oct. 25	52	Nov. 21—Dec. 11	Nov. 28
	Nov. 25	32	Dec. 4—Dec. 31	Dec. 14
Oldenburg	Sept. 25	129	Nov. 8—Dec. 14	Nov. 19
	Oct. 25	122	Nov. 21—Dec. 31	Dec. 3
	Nov. 25	123	Nov. 24—Jan. 10	Dec. 18
Versailles	Sept. 25	67	Nov. 15—Dec. 19	Nov. 28
	Oct. 25	63	Nov. 19—Jan. 5	Dec. 12
	Nov. 25	66	Dec. 10—Jan. 9	Dec. 23

Similarly, a temperature of 1.5° C stopped pupal development early in August but not early in November; in the latter case adults eventually emerged.

The conclusion of KOZHANCHIKOV (1950), that there is an inverse relation between pupal development rate in the northern U.S.S.R. and temperature over a considerable temperature range, is therefore correct for the total pupal period but does not apply continuously. On the other hand JANCKE's (1937) conclusion that temperature does not affect the duration of the pupal stage is not supported by the results of the present investigation. The basis of JANCKE's conclusion, that field records showed no relation between mean temperature during the pupal period and the first date that moths emerged, is inadequate because, for example, moths would emerge unusually late if the temperature was unusually low in summer and high in autumn, though it averaged the same as in a year in which both summer and autumn temperatures were optimal for pupal development. Though frequently it is impossible to associate time of emergence with the mean temperature during the pupal period, it is often possible to correlate it with intervals of unusually high or low temperature, as was done by FAES, STAEHELIN & BRUDERLEIN (1924) and PAILLOT (1934).

Whether development is usually continuous could be determined by studying sections taken from pupae at frequent intervals. Indirect evidence for uninterrupted development was obtained by SPEYER (1938b) who recorded a continuous decrease in pupal weight throughout the pupal stage. On the other hand, GRISON & SILVESTRE DE SACY (1948) believed that diapause occurred during summer because they observed no effect of temperature or soil moisture at that time on the dates that moths emerged. Their observation on temperature is, however, at variance with the results I obtained. MASSEE (1947) suggested that occasionally some winter moths do not emerge until the second autumn, that is until approximately 18 months after pupation, but did not suggest a cause for such delays. No evidence to support Massee's theory was found from rearings and dissections of many thousand pupae from different localities. Temperature extremes stop development and, if prolonged, cause much pupal mortality. This occurred in summer both in pupae held at 25° C by KOZHANCHIKOV (1950), and at 1.5° C in the present study. In the latter case higher temperatures, commencing during the following May, permitted development and female moths emerged from a small proportion of the pupae in August, that is, about eight months later than usual. Temperatures below approximately 0° C late in autumn prevent emergence and probably delay completion of pupal development but, even if prolonged, do not adversely affect females (WYLIE, 1960b). This probably accounts for records (SCHREINER, 1916; MACHOTIN, 1936) of moth emergence during spring in localities where winter temperatures are very low. However, low temperatures in autumn and winter probably did not cause the emergence of both males and females in February and March in southern England and south-eastern France, recorded by THEOBALD (1926) and PAILLOT (1923) respectively; but low temperatures late in autumn, preceded by unusually high temperatures in summer or early autumn, could have been responsible.

Soil Moisture

Emergence, at 6.5° C and 80 to 85 per cent relative humidity, was compared from two lots of pupae reared, from August 7 to October 25, in moist vermiculite and in vermiculite at a relative humidity of 60—65 per cent. The mean emergence dates of females from Versailles reared in the two conditions were December 7 and December 11, respectively; those of females from Oldenburg, November 30 and December 4. This supports the conclusion of REIFF (1953) that drought in summer delays adult emergence. REIFF also reported that, experimentally, unusually high soil moisture reduced the rate of pupal development and thereby delayed onset of emergence. JANCKE (1937) reported no relation between the total amount of rain during the pupal period and the date the first moths emerged; however, this does not prove that the amount of soil moisture has no effect on pupal development. Emergence would be delayed if pupae were exposed successively to opposite extremes of soil moisture but not if the same total amount of rain was more evenly distributed in time throughout the pupal period and extremes of soil moisture thereby avoided.

INTRINSIC FACTORS

Effects on the Eggs

The incubation period of eggs from Oldenburg is longer, at various temperatures, than that of eggs from Versailles (Table I). This suggested that the spread of over five months in the incubation period between eggs from different localities in Europe was caused by intrinsic differences in the eggs. To test this hypothesis, all the eggs laid by several females from Frankfurt, Oldenburg, and Versailles, which had copulated with males of equal age from the same locality, were incubated at 6.5° C and a relative humidity of 80—85 per cent. The incubation periods are shown in Table III.

TABLE III

Incubation periods at 6.5° C and relative humidity 80—85 per cent of eggs laid by females from different localities in Europe

Locality	Female	Number of Eggs	Incubation period of progeny (days) Mean	Standard deviation	Incubation period for population (days) Range	Mean	Standard deviation
Frankfurt	1	192	114	7.8			
	2	317	120	6.6			
	3	92	123	10.0	96—150	120	10.0
	4	90	131	9.5			
	5	43	118	16.0			
Versailles	1	174	128	5.2			
	2	259	139	4.6			
	3	66	131	6.7			
	4	177	131	5.0	115—162	137	8.5
	5	165	144	8.4			
	6	13	150	4.4			
	7	59	132	6.0			
	8	176	144	4.0			
Oldenburg	1	20	154	10.9			
	2	92	168	13.7			
	3	131	160	7.8			
	4	194	160	6.2	129—189	162	9.2
	5	18	176	6.0			
	6	105	163	9.1			
	7	112	162	4.8			
	8	106	169	6.9			

The incubation periods of eggs from the three localities differed (Tables I and III). This evidence supports the conclusion of SPEYER (1938a) who studied eggs from seven localities in Germany, Sweden and Switzerland. SPEYER's data also indicate that differences in the duration of the egg stage are inherent because he recorded the same sequence each year in times of moth emergence (which corresponds closely to onset of oviposition) and of larval hatch, when each "race" was inbred for several generations in the same environment. The present conclusion is at variance with that of SILVESTRI (1941), based on general field observations, that differences in the incubation periods in several localities in Italy were caused entirely by temperature differences.

The considerable variation (Table III) among the mean incubation period of the progeny of different females from one locality is attributed to intrinsic differences between the eggs laid by the different females. The relatively low incubation temperature (6.5° C), compared to that prevalent in nature late in the egg stage, may have magnified the variations but would not affect the relation among the means of different families. Though their own relatively early or late emergence has no effect, both parents influence the incubation periods of their progeny: this is indicated by the incubation periods (Table IV), of eggs laid by females from Versailles and Oldenburg that had been cross-fertilized by males of the same age.

TABLE IV

Effect of interbreeding moths from Versailles and Oldenburg on incubation periods of their progeny

Cross	Female	Number of Eggs	Incubation period of progeny (days)		Incubation period for cross (days)		
			Mean	Standard deviation	Range	Mean	Standard Deviation
Versailles ♂ × Oldenburg ♀	1	55	146	10.2	101—170	141	12.3
	2	80	141	6.6			
	3	130	143	6.4			
	4	65	127	8.9			
	5	253	148	4.9			
	6	204	153	4.9			
	7	34	145	6.5			
	8	126	152	6.2			
	9	144	134	6.4			
	10	177	135	9.2			
	11	23	145	5.6			
	12	45	158	4.6			
	13	194	122	4.3			
Oldenburg ♂ × Versailles ♀	1	208	159	4.9	115—173	154	9.6
	2	69	154	4.5			
	3	106	155	6.1			
	4	178	145	14.0			
	5	145	153	7.3			
	6	175	157	5.9			

Comparison of the data in Tables III and IV indicates that the incubation periods of the progeny of the crosses were affected by both parents and were intermediate between those of individuals from the two parent populations. This agrees with the conclusion of SPEYER (1941) that the progeny of a cross between early hatching and late hatching "races" of winter moth had intermediate hatching dates.

Intrinsic differences in the incubation period exist between individuals of each family (Table III). Similar variations were recorded in nature: BRIGGS (1957) showed that, in numerous families, eggs laid over a three or four day period and incubated in the field hatched over a much longer interval. In this case temperature differences in the environment of different eggs in each family were negligible because the oviposition period for each family was very short; therefore, intrinsic differences among the individuals are indicated.

Effects on the Pupae

Winter moths from different localities emerged at different times in the laboratory though the pupae were reared in the same environment (Table II). The additional data on female emergence, shown in Table V, were compiled from laboratory rearings in 1955 and 1956.

TABLE V

Emergence data for winter moth females from different localities that were reared as pupae in identical conditions

Year of Collection	Locality	Mean pupation date (app.)	Female emergence Number	Range	Mean	Mean duration of pupal stage (app.)
1955	Hasselt - Belgium	—	2090	Nov. 26—Dec. 29	Dec. 10	—
	Oostmalle - Belgium	—	1643	Nov. 12—Dec. 30	Dec. 5	—
	Frankfurt - Germany	May 31	2088	Nov. 14—Dec. 29	Dec. 1	184
	Oldenburg - Germany	June 20	3781	Nov. 18—Dec. 31	Dec. 3	166
	Versailles - France	May 31	1989	Nov. 19—Jan. 10	Dec. 15	198
	Wytham - England	June 8	658	Nov. 22—Jan. 12	Dec. 17	192
1956	Frankfurt - Germany	May 28	249	Nov. 19—Dec. 15	Dec. 3	189
	Oldenburg - Germany	June 10	2332	Nov. 16—Dec. 28	Dec. 6	179
	Versailles - France	May 31	1108	Nov. 18—Jan. 6	Dec. 13	196
	Kullaberg - Sweden	June 15	239	Nov. 22—Dec. 16	Dec. 4	172
	Oland - Sweden	June 13	394	Nov. 15—Dec. 21	Dec. 1	171

There was considerable variation between the mean dates of emergence for the different localities (Table V). The same sequence in the mean dates for the localities Frankfurt, Oldenburg, and Versailles, probably corresponding to the natural sequence of emergence, was recorded in both years. Late or early emergence in each locality cannot be correlated with late or early pupation because the durations of the pupal stage also differ. The extent to which these differences in pupal development were related to intrinsic differences in the pupae could be determined by inbreeding moths from discrete populations for several generations under identical conditions. Evidence for intrinsic differences was published by SPEYER (1938a) who recorded the dates of the onset of larval hatch and adult emergence of several "races", each of which had been inbred for several generations, and found that the same sequences in both hatch and emergence were maintained. His results indicate that natural differences in pupal stage duration were not eliminated when each population was inbred in the same environment and, therefore, are partly genetic. Subsequently he showed (SPEYER, 1941) that the progeny of moths whose emergence in the laboratory was respectively very early and very late had an intermediate emergence time.

SILVESTRI (1941) concluded that there were not consistent differences in emergence times of moths that had originated in four localities in Italy and Sicily and had developed in the same environment. However, his conclusion is invalid for he recorded that moths from all localities emerged during December and January only and did not calculate the mean emergence dates.

The rates of development of individuals from one locality also differ. This is shown by the long emergence period of moths from each area (Table V) which was caused only partly by the fact that the larvae in each locality had pupated over a period of approximately ten days.

DISCUSSION

Freshly-laid eggs develop for some time at —4.5° C but no larvae hatch below approximately 6.0° C. Between at least 6.0° C and 18° C high temperature increases the rate of egg development. Pupal development rate is, in general, reduced by higher temperatures, though the optimum temperature for rapid development decreases as the pupae mature: thus, 1.5° C arrests development in summer but not late in autumn. Because, in general, higher temperatures accelerate egg and prolong pupal development, the duration of the egg stage is decreased and that of the pupa increased in southern localities of Europe compared to northern ones. Temperature also indirectly affects the duration of the pupal stage. KOZHANCHIKOV (1950) showed that reduced food consumption by the larvae at various temperatures between 11° C and 25° C increased the duration of the pupal stage. The amount of food eaten by each larva is reduced both by higher temperatures, at least in the range 10° C—25° C (GRISON & ROEHRICH, 1949) and by high larval population densities that destroy all suitable foliage before the larvae mature. Therefore, both temperature and intraspecific competition during larval development influence the duration of the pupal stage.

Inherent differences in the egg and pupal stages from different localities increase the effect of temperature differences. The evidence obtained for inherent differences supports the conclusion of SPEYER (1938a) but not that of KOZHANCHIKOV (1950). Additional rearings would indicate the relative importance of climatic and intrinsic factors in causing differences in the annual cycle throughout Europe.

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RESUME

La température affecte directement le taux de développement des oeufs et inversement celui des pupes chez la phalène hiémale, *Operophtera brumata* (L.). Dans le nord de l'Europe, une température relativement basse a pour effet de prolonger la durée du stade de l'oeuf et de raccourcir proportionnellement celle du stade de la pupe, tandis que dans le sud de l'Europe avec de plus hautes températures, l'effet est contraire. Les différences inhérentes aux oeufs et aux pupes dans diverses localités augmentent l'effet des différences de température.

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UNTERSUCHUNGEN ÜBER DIE WIRKUNG VON INSEKTIZIDEN UND VON PFLANZENALKALOIDEN AUF DEN DARMTRAKTUS DES KARTOFFELKÄFERS UND SEINER LARVEN

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Es werden die Ergebnisse der Untersuchungen über die Wirkung der Alkaloide: Tomatin, Atropin, Nicotin, Colchicin, Veratrin und Physostigmin, sowie der Insektizide: Arsenik, Wofatox (Thiophosphorsäureesterpräparat) und Certoxan (DDT-Präparat) auf den Darmkanal und auf das Verhalten von Larven und Adulten des Kartoffelkäfers mitgeteilt und kurz besprochen.

Bei den an der Forschungsstelle für Kartoffelkäferbekämpfung in Mühlhausen laufenden Untersuchungen wurden in den letzten Jahren etwa 80 verschiedene Pflanzeninhaltsstoffe (vorwiegend Alkaloide) auf ihre Larvenwirksamkeit getestet (BUHR, TOBALL & SCHREIBER, 1958; SCHREIBER, 1957). Einige dieser Stoffe konnten für die vorliegenden Untersuchungen ausgenutzt und in ihrer Wirkung auf den Darmkanal mit jener von drei industriell hergestellten Insektiziden verglichen werden. Unsere Darstellung behandelt zunächst die Wirkung der genannten Insektizide, anschließend die Ergebnisse von Untersuchungen über die Wirksamkeit von Pflanzen-Alkaloiden, die in den Substraten des Kartoffelkäfers (*Leptinotarsa decemlineata* Say) nicht vorkommen und schließlich die mit Solanaceen-Alkaloiden erzielten Befunde.

MATERIAL UND METHODE

Die betreffenden Substanzen wurden auf Laub der Kartoffelsorte Aquila entweder dünn aufgetragen oder infiltriert. Einige Alkaloide wurden nach der Methode von KUHN & GAUHE, (1947) in Gelatine gelöst aufgestrichen. Alle später angegebenen Konzentrationen beziehen sich auf das Blattfrischgewicht. Die Versuchstiere entstammten den Kartoffelkäferzuchten der Forschungsstelle für Kartoffelkäferbekämpfung Mühlhausen; verwendet wurden sämtliche Larvenstadien (L_1 - L_4) sowie Imagines. Für histologische Untersuchungen bestimmte Tiere fixierten wir vornehmlich in „Susa" oder „Carnoy" (ROMEIS, 1948) und überführten sie nach der Methylbenzoat-Benzol-Methode in Paraffin. Die 3 - 10 μ dicken Schnitte wurden in den meisten Fällen nach dem Azan-Verfahren gefärbt. Untersucht wurden das Verhalten der Tiere nach Aufnahme der verschiedenen

Substanzen sowie die mikroskopisch wahrnehmbaren Veränderungen im Darmepithel von Larven und Adulten.

DIE WIRKUNG VON INDUSTRIELL HERGESTELLTEN GIFTEN

Arsenik

Arsenik wirkt im tierischen Organismus lokal zellzerstörend, es hemmt die Funktion verschiedener Fermente und beeinflußt die Reduktions- und Oxydationsvorgänge in den Zellen. Für Insekten wirken arsenhaltige Mittel als Fraßgifte, welche vornehmlich das Mitteldarmepithel schädigen (PILAT, 1935; STEINHAUS, 1949; WILSON, 1936; WOKE, 1940). Wir verwendeten eine ca. 1 %ige wässrige Lösung von Arsenik, die entweder auf dem Kartoffellaub dünn aufgesprüht oder in anderen Fällen infiltriert wurde.

Larven wie Käfer nahmen derart zubereitetes Futter anfänglich normal auf. Eine abweisende (repellent-) oder vergällende (lymanent-) Wirkung (BUHR, in litt.) dürfte dem Arsenik daher nicht zukommen [1]). Nach etwa 10 - 20 Minuten normalen Fressens unterbrechen die Tiere die Futteraufnahme und zeigen Verkrampfungen. Mitunter werden diese Krampfzustände von heftigen Bewegungen unterbrochen, wobei die Larven bisweilen kleine Strecken hastig laufen. Die Adulten spreizen die Hinterbeine steif auseinander, ziehen den Kopf ein und verharren in dieser Haltung kürzere oder längere Zeit. Männliche Tiere unternehmen gelegentlich Kopulationsversuche, ohne jedoch das Genitalorgan zu entfalten. Bei den angewendeten Konzentrationen trat der Tod bei den Larven überwiegend bereits 75 Min. nach der Giftaufnahme ein. Bei den Adulten vergingen bis zum Exitus mindestens 2 Stunden, in der Regel eine noch längere Zeit.

Im histologischen Bild (Abb. 1 u. 2) erkennt man, daß die Schädigung des Darmepithels von Larven und Käfern durch Arsenik mit Schrumpfungen und Schaumigwerden des Protoplasmas beginnt, in den Kernen mit einer Verklumpung des Chromatins (Abb. 3). Die Darmmuskulatur kontrahiert sich mehr oder weniger. Später werden, zunächst einzelne Zellen, dann ganze Stücke des Darmepithels in das Lumen hinein abgestossen, Basalmembran und Muscularis bleiben dabei erhalten. Bei dem Eintritt des Todes befindet sich das Epithel des vorderen und mittleren Teiles vom Mitteldarm in vollkommener Zerstörung und liegt in Bruchstücken im Darmlumen (Abb. 4). Zuerst werden offenbar die resorbierenden Zellen (KOCH, in litt.) im mittleren und vorderen Teil des Mitteldarms geschädigt, später wenn hier die Epithelauflösung schon im Gange ist (2 bis 10 Stunden nach der Giftaufname), sind die überwiegend sekretorisch tätigen Zellen im analwärtigen Teil des Mitteldarms noch einigermaßen intakt. Ähnlich wie bei verschiedenen anderen Insekten (PILAT, 1935) bleiben diese Zellen auch nach längerer Gifteinwirkung noch im Epithelverband und lösen sich nicht von der Basalmembran. Sie werden offenbar erst nach dem Tode des Individuums zerstört. Unter dem Einfluss des Arsenik scheint anfänglich eine Hypersekretion einzusetzen. Im apikalen Zellraum und im Lumen liegen zahlreiche Sekretblasen, die den Streifensaum von vielen Zellen abgehoben und teilweise zum Platzen gebracht haben. Im Enddarm treten verschiedene Veränderungen — frühestens nach einer

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[1]) Anmerk.: Hervorgehoben sei, daß Kartoffelblätter, welche in gleicher Weise mit einer wässrigen Lösung von Sublimat bestrichen oder infiltriert wurden, zwar betastet, nicht aber angebissen werden (deterrent-Wirkung).

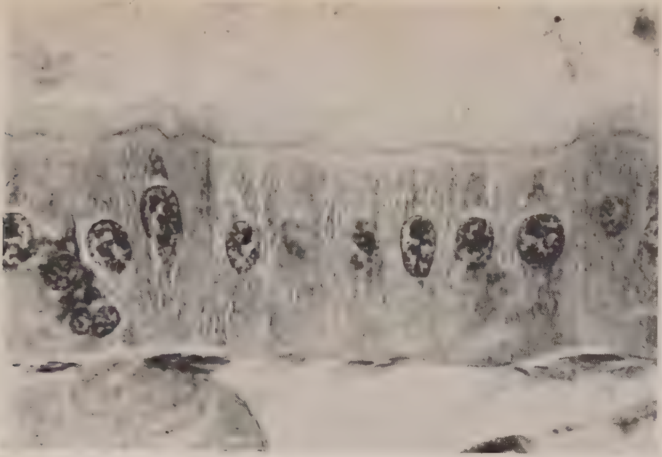

Abb. 1. Mitteldarmepithel einer gesunden Larve; links ein Regenerationsnest (8 μ; Azan: 700 ×)

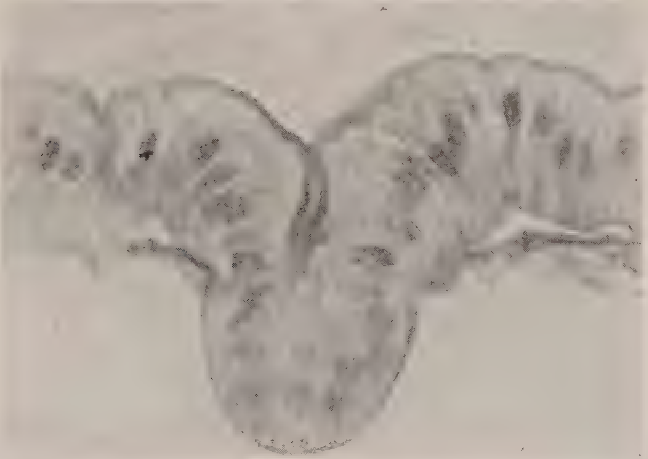

Abb. 2. Mitteldarmepithel eines gesunden Käfers, in der Mitte eine Regenerationskrypte (7,5 μ; Azan; 500 ×)

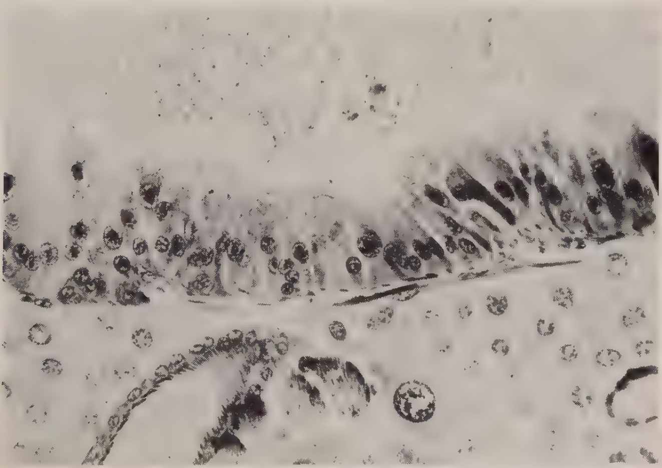

Abb. 3. Mitteldarmepithel einer Larve, 1,5 Stunden nach Arsenikaufnahme, Beginn der Epithelzerstörung (7 μ; Azan; 360 ×)

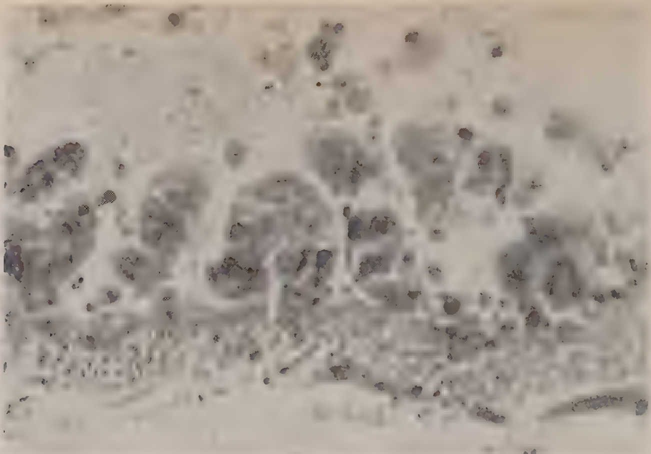

Abb. 4. Mitteldarmepithel einer Larve, 15 Stunden nach Arsenikaufnahme, Epithel vollständig zerstört (7,5 μ; Azan; 360 ×)

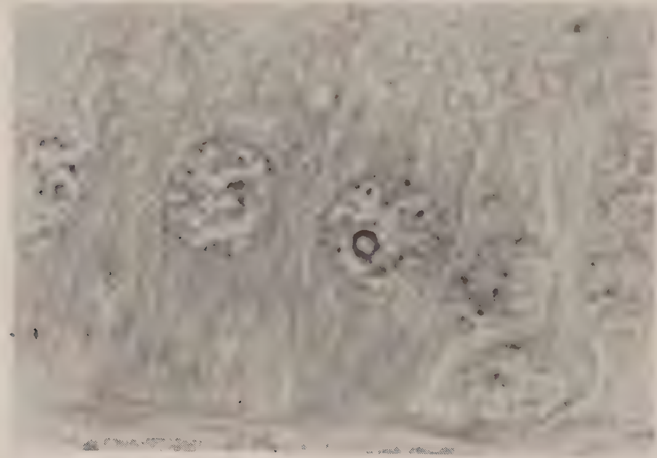

Abb. 5. Mitteldarmepithelzellen (Ausschnitt) einer Larve nach DDT Vergiftung. Im Kern der mittleren Zelle ist die Vakuole im Nucleolus erkennbar. (6 μ, Azan; 1700 ×)

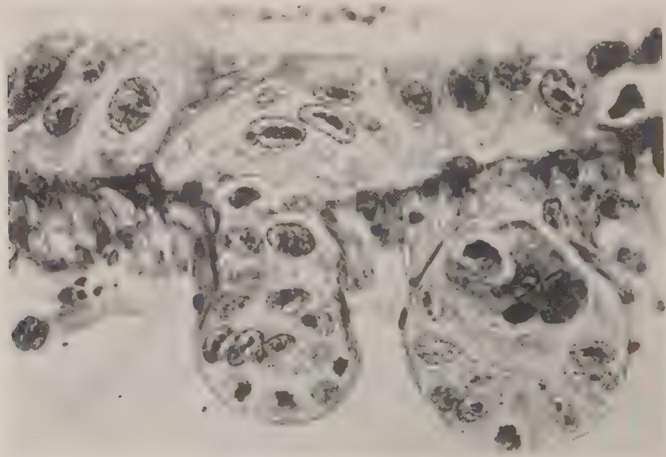

Abb. 6. Mitteldarmepithel eines Käfers nach Colchicinaufnahme, Beginn der Epithelauflösung. In den Regenerationskrypten mehrerer Mitoseanomalien (Verklumpungen in Metaphasestadium). (7 μ; Azan; 500 ×)

Stunde — nach der Gifteinnahme auf. Das Plasma der Zellen im Colon und Ileum scheint sich zu verdichten, wobei einzelne Vakuolen unterschiedlicher Größe auftreten. Die Zellkerne werden pyknotisch.

Arsenik schädigt in erster Linie Protoplasmakomponenten, während die Kerne erst sekundär angegriffen werden. Obwohl dem Arsenik eine geringe Ätzwirkung zukommt, dürfte die Epithelauflösung nicht auf eine direkte Wirkung dieses Giftes zurückgehen, sondern auf Selbstverdauungsvorgängen beruhen.

Certoxan und Wofatox

Die Kontaktinsektizide Certoxan ¹) (DDT-Präparat) und Wofatox ¹) (Thiophosphorsäureester-Präparat) wurden in wässriger Verdünnung (Certoxan 0,5 %; Wofatox 0,03 %) auf die Kartoffelblätter aufgesprüht. Wegen der unterschiedlichen Giftempfindlichkeit der einzelnen Larvenstadien (LANGENBUCH, 1955) wurden für diese Versuche nur Larven im 2. oder 3. Stadium verwendet.

Über die physiologischen Wirkungen von organischen Insektiziden der genannten Wirkstoffgruppen besteht noch keine entgültige Klarheit. Es scheint erwiesen, daß derartige Substanzen nicht ausschließlich bestimmte Organe schädigen, sondern durch ihre lipophilen Eigenschaften in das Gefüge der Zellipoide verändernd eingreifen, dadurch zu schweren Stoffwechselstörungen führen und schließlich den Exitus bewirken können (JOCHUM, 1956).

Certoxan. Larven und Käfer beginnen, ohne zu zögern, das vergiftete Futter zu fressen. Bereits nach wenigen Minuten wandern die ersten Tiere ab, gleichlaufend stellt sich vorübergehend eine Hyperaktivität ein, die nach mehreren Minuten in Krampfzustände unterschiedlicher Intensität übergeht. Die Larven beginnen die Beine abzustrecken, gehen kurze Strecken rückwärts und erheben den Vorderkörper, wobei sie die typischen Abwehrbewegungen mit dem vorderen und mittleren Beinpaar ausführen. Dann verharren sie ruhig mit eingezogenen Abdomen und weit abgestreckten Beinen. Von diesen anfänglichen Verkrampfungen können sich die Tiere scheinbar vorübergehend wieder erholen, doch nehmen sie in der Regel anschließend keine Nahrung auf. Nach 16 Minuten fielen die ersten Larven in Rückenlage und nach etwa einer Stunde waren fast alle Versuchstiere verendet.

Die Imagines verlieren die Gewalt über einzelne Extremitäten und sitzen schließlich ruhig da, die Vorderbeine angezogen, die Fühler angelegt, die Hinterbeine steif abgestreckt, so daß das Abdomen aufwärts gerichtet ist. Die ersten Käfer mit Rückenlage wurden nach 17 Minuten beobachtet; einem Teil von ihnen gelang es, sich vorübergehend wieder aufzurichten. Nach 75 Minuten waren fast alle Tiere ohne Bewegung und in Rückenlage. Der Tod trat im Laufe der nächsten Stunden ein (vgl. JOCHUM, 1956).

Bei den histologischen Untersuchungen des Darmkanals zeigten sich mehrere gut ausgebildete Symptome. Das Protoplasma der aktiven Mitteldarmepithelzellen verquillt und wird zwischen Kern und Streifensaum schaumig. Die Kerne dieser Zellen sind stark vergrößert, ohne daß zunächst Chromatinverklumpungen auftreten. Die Nucleolen in solchen Kernen sind angeschwollen und enthalten eine,

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¹) VEB Farbenfabriken Wolfen/Krs. Bitterfeld.

bei gesunden Tieren nicht vorkommende (KOCH, in litt.) rundliche Vakuole (Abb. 5). Die Regenerationszellen werden durch DDT nicht sichtbar geschädigt. Selbst eine Stunde nach der Giftaufnahme finden sich noch verschiedene Mitosestadien. Im analen Teil des Mitteldarmes, in dem nach der Giftaufnahme eine starke Hypersekretion einsetzt, sind die Vergiftungssymptome weniger deutlich ausgeprägt als in den voraufgehenden Abschnitten. Diese Beobachtung steht im Einklang mit Befunden von JOCHUM (1956), der beim gleichen Objekt nach E 605-Vergiftung einen starken Schwund der Leibeshöhlenflüßigkeit und eine entsprechende Zunahme des Darmvolumens beobachtete.

Der Epithelverband wird selbst längere Zeit nach dem Eintreten der Rückenlage nicht aufgelöst, auch unterbleibt eine verstärkte Ausstossung von Zellen in das Darmlumen, ein Vorgang, der bei anderen Insekten beobachtet worden ist (HOPP, 1953). Bei Adulten und Larven beginnt die Auflösung des Mitteldarmepithels vermutlich durch Selbstverdauungsvorgänge erst nach dem Tode. Dabei färben sich die abgestorbenen Tiere dunkel und verjauchen in kurzer Zeit vollständig.

Im Enddarm fehlen deutliche Vergiftungssymptomen, obwohl sich gelegentlich verklumpte Kerne sowie Vakuolen im Plasma fanden. Offen bleibt, ob diese Schädigungen ihren Ausgang vom Darmlumen oder von der Leibeshöhle her nehmen.

Wofatox. Nach Darbietung von Phosphorsäureesterpräparaten ähneln die Vergiftungssymptome jenen, wie sie nach DDT-Vergiftung beobachtet wurden, jedoch äußern sie sich weniger heftig. Zwischen den Krampfphasen nehmen die Tiere meist wieder Nahrung auf, Rückenlage und Tod treten erst später ein. Auch in histologisch-cytologischer Hinsicht besteht zwischen den mit Wofatox und den mit Certoxan vergifteten Larven und Käfern eine weitgehende Übereinstimmung. Die Intensität der Sekretabgabe steigert sich und das Plasma verschiedener Mitteldarmzellen wird schaumig. In vielen Zellen finden sich vergrößerte Kerne, deren Nucleolus aufgetrieben ist und ebenfalls eine kleine rundliche Vakuole (vgl. Abb. 5) enhält. Eine Giftwirkung ist hauptsächlich in den aktiven Zellen des mittleren und vorderen Teils vom Mitteldarm festzustellen. Die Regenerationszellen scheinen weniger geschädigt zu werden. Ein Zerfall des Epithels im Mitteldarm setzt erst nach dem Tode ein und dürfte nicht auf eine direkte Insektizidwirkung zurückgehen. Diese gleichartige Wirkung von DDT und Thiophosphorsäureester-Präparaten scheint zu bestätigen, daß sich die lipophilen Kontaktinsektizide in ihren physiologischen Wirkungen nicht grundsätzlich unterscheiden, sondern auf ähnliche Weise in den Zellstoffwechsel schädigend eingreifen.

DIE WIRKUNG VON PFLANZENINHALTSSTOFFEN

Colchicin

Colchicin, das Hauptalkaloid aus *Colchicum autumnale* L. besitzt weder für Larven noch für Imagines eine abschreckende oder vergällende Wirkung. In Konzentrationen von 0,1 - 0,5 % wirkt es bereits stark toxisch; schon in sehr geringen Konzentrationen (z.B. 0,002 %) verhindert es eine normale Entwicklung und führt im Verlaufe von 20 Tagen zum Absterben aller Versuchstiere.

Larven und Käfer nehmen bis zum Einsetzen der ersten Vergiftungserscheinungen die Colchicin-versetzte Nahrung (0,1 - 0,5 %) normal auf. Nach 8 - 10

Min. unterbrechen sie den Fraß, um ruhig zu verharren oder auch kurze Strecken hastig zu laufen. Die Larven bleiben, oft bis zum Eintritt des Todes, an der gleichen Stelle sitzen und geben nur hin und wieder dünnen Kot ab. Im vorgeschrittenen Vergiftungsstadium sind sie aufgedunsen, ihr Abdomen ist ventralseitig mit Kot und Exkreten verschmiert. Die Käfer beginnen etwa 10 Minuten nach der Giftaufnahme zu taumeln, wobei sie mitunter heftige, aber unsichere Bewegungen mit den Extremitäten ausführen. Ebenso wie nach Arsenikaufnahme stellen die Männchen gelegentlich Kopulationsversuche an, ohne aber den Genitalapparat zu entfalten. Später kann Rückenlage eintreten, aus der sich die Tiere oft nicht mehr erholen, bis zum Exitus vergehen jedoch meist mehrere Stunden.

Beim Auftreten der ersten äußeren Vergiftungssymptome erscheinen die Mitteldarmepithelzellen völlig unverändert. Etwa 20 Minuten nach der Colchicinaufnahme wird das Plasma zwischen Kern und Lumen schaumig; gleichlaufend setzt eine verstärkte Sekretabgabe ein. Bei vielen aktiven Zellen, offenbar den Sekretzellen, vergrößert sich der Nucleolus deutlich, wobei jedoch eine Vakuolenbildung unterbleibt. Die Färbbarkeit des Chromatins mit Azokarmin geht zurück, der Nucleolus tritt dadurch noch stärker hervor. Später läßt auch die Reaktionsfähigkeit der DNS mit dem Thionin-SO_2-Reagens (VAN DUIJN, 1956) an Intensität nach. Zur gleichen Zeit setzt, besonders im vorderen und mittleren Teil des Mitteldarms, eine verstärkte Ausstossung von Zellen in das Darmlumen ein. Im Lumen bleiben diese Zellen kurzfristig intakt und werden dann aufgelöst. Die durch die ausgestossenen Zellen entstandenen Lücken werden anfangs durch Verbreiterung der im Epithelverband stehenden, sich dabei verkürzenden Zellen geschlossen. Dann beginnt die Auflösung des gesamten Darmepithels, die schließlich auch die Regenerationszellen erfaßt. Beim Eintritt des Todes ist der Zellverband bis auf wenige Reste zerstört. Im Gegensatz zu der nach Arsenikgaben beobachteten Auflösung, lösen sich nach Colchicineinwirkung in der Regel nicht ganze Epithelstücken, sondern einzelne Zellen ab. In den Regenerationszellen beginnt etwa eine halbe Stunde nach der Colchicinaufnahme die Zahl der Mitosen erheblich zuzunehmen. Diese Mitosen gehen jedoch über das Metaphasestadium nicht hinaus und verklumpen schließlich (Abb. 6). Auch in weiteren Geweben (Hypodermis, Follikelzellen, Hodenzellen) nimmt die Zahl der ebenfalls bald degenerierenden Mitosen zu. Die Zellen der anderen Darmteile zeigen unter dem Einfluß von Colchicin primär keine Veränderungen. Im vorgeschrittenen Vergiftungsstadium, wenn im Mitteldarm die Epithelauflösung beginnt, werden im Enddarm die Kerne pyknotisch und degenerieren schließlich; im Plasma waren charakteristische Symptome nicht erkennbar.

Veratrin

Veratrin ist ein Alkaloidgemisch (Cevadin und Veratridin) aus *Schoenocaulon officinale* (Schldl. et Cham.). Es ist als Insektizid (Sabadillessig) bekannt (VELBINGER, 1947) und wirkt vor allen muskeldepolarisierend und schleimhautreizend. Wir verabreichten Veratrin in etwa 0,3 %iger Konzentration mit der Nahrung. Es wirkt auf Larven und Käfer stark toxisch. Während die Larven mit Veratrin versetzte Kartoffelblätter meist sofort angreifen, beginnen die Adulten nur zögernd zu fressen und nehmen nur wenig oder überhaupt kein Futter auf. Etwa 2 Minuten nach dem Veratringenuß treten bei den Larven die ersten Verkrampfungen auf. Der Gang wird schleppend, die Tiere bäumen sich auf und verlieren das Gleichgewicht, worauf Rücken- oder Seitenlage eintritt. Meist können sie sich nicht wieder aufrichten; sie ziehen das Abdomen ein und geben gelegentlich aus Mund und After eine braune Flüßigkeit ab. Der Tod tritt innerhalb von 2 - 3 Stunden ein; die verendeten Larven sind vollständig erschlafft.

Die Imagines zeigen schon innerhalb weniger Minuten die ersten Verkrampfungen, wobei die Antennen angelegt, die Vorderbeine angezogen und die Hinterbeine steif abgestreckt werden. Wenn sich solche Käfer bewegen, werden die Beine, offenbar aufgrund der muskeldepolarisierenden Veratrinwirkung, nur schleppend vorangesetzt. Später fallen die Käfer in Rückenlage, aus der sie sich normalerweise nicht wieder erheben. Der Tod tritt im Laufe der folgenden Stunden ein. Veratrin zeigt eine starke Kontaktwirkung, denn auch Tiere, die mit dem behandelten Futter längere Zeit nur in Berührung gekommen sind, und es dabei mit Tarsen, Tastern oder den Mundwerkzeugen berührten, gehen zugrunde.

Im Mitteldarm verursacht Veratrin die Ausstossung von zahlreichen Epithelzellen in das Darmlumen sowie eine Steigerung der Sekretabgabe. Beim Auftreten der ersten Verkrampfungen ist der Streifensaum der Mitteldarmepithelzellen an vielen Stellen blasenförmig aufgetrieben, weitere pathologische Veränderungen könnten nicht festgestellt werden.

Physostigmin

Physostigmin (Eserin) ist ein Alkaloid aus *Physostigma venenosum* Balfour, das vor allem als Cholinesterasehemmer bekannt ist. Mitteilungen über die Wirkung von Physostigmin auf den Insektenorganismus liegen bisher nicht vor. Wir benutzten Physostigmin in Lösungen, die im behandelten Kartoffellaub zu einer 0,3 %igen Konzentration führten. Ähnlich wie Veratrin-Futter wird auch das mit Physostigminlösungen bestrichene Futter von den Larven sofort, von den Adulten dagegen nur zögernd gefressen. Die Larven zeigen nach etwa 5 bis 10 Minuten Fraß Verkrampfungen. Die Käfer legen nach Physostigmingenuß die Antennen an, strecken die Beine steif aus und spreizen gelegentlich die Elytren weit ab. Zeitweilig führen die Tiere heftige Bewegungen aus und verharren dann längere Zeit ruhig. Der Tod tritt bei Larven und Käfern nach einigen Stunden ein. Da Imagines, die Physostigmin-versetztes Futter nicht aufgenommen, es aber berührt und betastet hatten, Verkrampfungen zeigten und z. T. zugrunde gingen, dürfte auch diesem Alkaloid eine Kontaktwirkung zukommen. Nach dem Genuß von Physostigmin konnten weder bei Larven noch bei Käfern histologisch oder cytologisch sichtbare Veränderungen nachgewiesen werden.

Nicotin

Nicotin, das Hauptalkaloid verschiedener *Nicotiana*-Arten ist als Insektizid gebräuchlich. Es wirkt vor allem schädigend auf das Nervensystem (Lähmung der Synapsen), ferner führt es nach höheren Dosen zu baldigem Herzstillstand (ROEDER, 1953; STEINHAUS, 1949). Auf den Kartoffelkäfer und seine Larven wirkt Nicotin stark toxisch und für die Adulten offenbar in geringem Maße Futtervergällend. Die nicotinreichen Tabakarten (*Nicotiana tabacum* L. und *N. rustica* L.) sind wegen ihres hohen Nicotingehaltes voll resistent (BUHR, 1956; CHIN, 1950; KOENIG & KOELLE, 1950). Verschiedene nicotinarme Ziertabake (z.B. *N.*

sanderae Hort., *N. affinis* T. Moore) ermöglichen eine, wenn auch gehemmte Larvenentwicklung (BUHR, 1956).

Wird Nicotin in Lösungen von 0,5 % und höher nach einem der eingangs beschriebenen Verfahren auf das Kartoffellaub aufgebracht, so wirkt es sofort stark toxisch. Bereits nach dem ersten Fraßversuch bäumen sich die Tiere auf, geben aus Mund und After eine bräunliche Flüßigkeit ab, verkrampfen die Extremitäten und rollen sich ein. Der Tod tritt ,,blitzartig'' innerhalb kürzester Fristen ein. Eine derartig rapide Wirkung kann nicht über resorbierende Mitteldarmepithelzellen erfolgt sein, findet sich doch die Nahrung in so kurzer Zeit noch in der Buccalhöhle; vielmehr dürfte die Vergiftung durch eine Kontaktwirkung zustande kommen. Pathologische Veränderungen des Darmepithels sind deshalb nicht zu erwarten, wie durch die Untersuchungen bestätigt wurde. Schwächere Nicotinkonzentrationen (0,003 %) in der Nahrung wirken nicht akut toxisch, sondern werden längere Zeit vertragen und gestatten die Entwicklung einzelner Larven. Diese nehmen solches Futter ungern an und fressen nur periodisch kleine Mengen. Es können leichte Verkrampfungen auftreten, die aber meist bald abklingen. Danach wandern die Tiere in der Regel vom Futter ab und hungern einige Zeit. Auch bei solchen Larven bzw. Käfern konnten pathologische Veränderungen des Darmepithels nicht gefunden werden, lediglich die Zahl der ausgestossenen Zellen zeigte einen geringen Anstieg.

Atropin

Atropin ist das Hauptalkaloid aus der Tolkirsche, *Atropa belladonna* L., deren heimische Freilandformen für Käfer und Larven anfällig sind (BUHR, 1956). In Fütterungstesten mit relativ geringen Mengen des Alkaloids auf Kartoffellaub, ergeben sich zunächst keine Beeinträchtigungen der Larvenentwicklung, später resultiert eine erhöhte Sterblichkeit im L_4-Stadium, auch scheint es, daß die weitere Metamorphose gehemmt wird (SCHREIBER, 1957). Larven und Käfer, die mit Tollkirschenblättern gefüttert wurden, wiesen im Darmepithel keine Anomalien auf. Auch bei Fütterungsversuchen mit Alkaloid-Konzentrationen, die dem Atropingehalt der Tollkirschenblätter (0,4 - 0,9 %) annähernd entsprechen, wurden Veränderungen nicht festgestellt. Ein Einfluß des Atropins auf die Sekretionstätigkeit konnte nicht nachgewiesen werden. Erst bei Erhöhung der Atropinkonzentration auf 3 - 6 % stellt sich neben einer vergällenden auch eine toxische Wirkung ein. Die Tiere fressen nur wenig, bereits kurze Zeit nach der Futteraufnahme treten Verkrampfungen ein. Einige Larven, die nur wenige Quadratmillimeter des Futters aufgenommen hatten, waren bereits nach 2 Stunden verendet. Die Adulten verweigern im allgemeinen die Aufnahme solchen Futters; Käfer, die dennoch, wenn auch in geringen Umfange davon frassen, zeigten zwar vorübergehende Verkrampfungen, blieben aber normalerweise am Leben.

Die histologische Untersuchung des Darmes von Larven ergibt, daß nach Genuß von hohen Atropindosen im mittleren und vorderen Teil des Mitteldarms das Plasma der Epithelzellen schrumpft und die Kerne pyknotisch werden. Nach 1 - 2 Stunden löst sich das Darmepithel auf und liegt dann in größeren Bruchstücken im Lumen; Basalmembran und Muscularis bleiben dabei erhalten.

Tomatin

Tomatin ist ein Glykosteroidalkaloid (Alkaloidglykosid, Glykoalkaloid), das in verschiedenen *Lycopersicon*-Arten vorkommt (KUHN, LÖW & GAUHE, 1950; PROKOSCHEW, PETROTSCHENKO & BARANOVA, 1952; SCHREIBER, 1954, 1956). Neuerdings wurde es von SCHREIBER (1959) auch in einigen Wildkartoffeln (z.B. *Solanum polyadenium* Greenm.) und überraschenderweise auch in verschiedenen Herkünften von *Solanum demissum* Lindl., in denen es sogar das Hauptalkaloid darstellt, nachgewiesen.

Erwiesen ist, daß die Tetraoside unter den Solanaceen — Glykoalkaloiden wie z.B. Tomatin und sicher auch Demissin als resistenzbedingende Prinzipien verschiedener *Lycopersicon* bzw. *Solanum*-Arten anzusehen sind (BUHR, 1956; BUHR, TOBALL & SCHREIBER, 1958; KUHN & GAUHE, 1947; SCHREIBER, 1958; STÜRKOW, 1959). Pflanzen, mit einem bestimmten Gehalt an solchen Alkaloiden werden nur zögernd als Futter angenommen und dann mit steigendem Alkaloidgehalt zunehmend resistenter. Eine Alkaloidkonzentration von etwa 0,45 - 0,5 % im Blattfrischgewicht genügt, um eine Larvenentwicklung weitestgehend zu verhindern, eine Wirkung, die den Triosiden dieser Stoffgruppe, etwa dem Solanin, nicht zukommt. In den eigenen Versuchen wurde Tomatin in 0,5 - 0,7 %iger und 0,8 - 1 %iger Konzentration, bezogen auf das Blattfrischgewicht, dargeboten. Derartiges Futter wird von der Mehrzahl der Larven und Käfer betastet und anfangs geringfügig befressen; daraufhin wandern die Tiere ab und greifen das Futter erst nach längerer Hungerpause wieder an. Die Menge der aufgenommenen Nahrung ist im allgemeinen gering, nur einzelne Individuen vertilgen dieses tomatinhaltige Futter in größerem Umfang. Im Vergleich zu entsprechend normal ernährten bzw. zu hungernden Kontrolltieren zeigen Larven und Käfer nach Tomatingenuß leichte Verkrampfungen. Während hungernde Tiere stets umherwandern, sitzen Individuen, die ebensolange hungern mußten, und dann tomatinhaltiges Futter aufgenommen hatten, mit eingezogenen Abdomen ruhig da; erst nach einiger Zeit (10 bis 20 Minuten) beginnen solche Tiere wieder umherzulaufen. Einzelne Tiere, die größere Mengen von tomatinhaltiger Nahrung aufgenommen hatten, erbrachen gelegentlich nach wenigen Minuten Teile des Darminhaltes. Bei den gebotenen Tomatinkonzentrationen gehen alle Larven nach einiger Zeit zugrunde. Im Vergleich mit hungernden Larven zeigte sich jedoch, daß die

TABELLE I

Durchschnittliche Gewichtsveränderungen in mg je Larve bei Junglarven des 2. Stadiums, die mit normalen, bzw. mit tomatinhaltigen Futter oder als Hungertiere gehalten wurden. Mittelwerte von Serien mit je 40 Tieren (Temperatur 24 ± 1° C; relative Luftfeuchtigkeit 92 bis 95%; 17 Stunden tägliche Beleuchtung).

Stunden nach Versuchsbeginn	Normalfutter	Tomatin-haltiges Futter		Hungertiere
		0,5—0,7%	0,8—1%	
0	3,23	3,23	3,23	3,23
4	3,60	3,16	3,11	3,01
24	4,77	3,12	2,96	2,77
28	5,21	3,36	2,92	2,74
48	7,37	3,39	2,75	2,53
52	7,65	4,07	2,78	2,43
72	10,04	4,28	2,72	2,11

Tomatin-Tiere durchschnittlich etwas länger am Leben bleiben als jene. Um die Gewichtsveränderungen von Larven, die mit normalen und Tomatinversetztem Blattmaterial gefüttert wurden, im Vergleich zu Hungertieren zu ermitteln, wurden jeweils Serien von 40 Larven im 2. Stadium gleichzeitig unter weitestgehend konstanten Bedingungen isoliert gehalten und täglich zweimal gewogen (Tab. I).

Daraus ergibt sich, daß die Larven am Tomatin-Futter im Durchschnitt entweder bedeutend weniger an Gewicht zunehmen als normal ernährte Tiere oder sogar an Gewicht verlieren. Die Gewichtsschwankungen der Larven an Tomatin-Futter (0,5 - 0,7 %) sind darauf zurückzuführen, daß diese Tiere nach anfänglicher Hungerzeit mehr Futter aufnahmen, als diejenigen, welche an Tomatinreicherem Futter (0,8 - 1 %) gehalten wurden. Bei Darbietung von Tomatin in der letztgenannten Konzentration nahmen einige Larven (ca. 15 %) — wohl infolge des Erbrechens — zu Versuchsbeginn sogar stärker an Gewicht ab als entsprechende Hungertiere, obwohl sie geringe Mengen des Futters aufgenommen hatten (Diese Abweichungen kommen in den Durchschnittswerten der Tabelle I nicht zum Ausdruck).

In unseren Versuchen wurde der Darm von Tieren, die tomatinhaltige Nahrung gefressen hatten, vor allem mit jenem hungernder Larven und Käfer verglichen. Dabei ergab sich, daß weder im Plasma noch im Kern der Mitteldarmzellen irgendwelche Anzeichen pathologischer Veränderungen feststellbar waren. Auch der Streifensaum, der nach Verfütterung von *Solanum demissum* stellenweise aufgelöst werden soll (CHIN, 1950), erwies sich gegenüber normal ernährten oder hungernden Tieren als nicht verändert.

Besonders wurde geprüft, ob nach Genuß von Tomatin Vakuolen in verstärktem Ausmaß gebildet werden. Unter normalen Bedingungen finden sich unterschiedlich große Vakuolen (Sekretvakuolen) regelmäßig in vielen Mitteldarmzellen (BUSNEL, 1939; KOCH, in litt.). Zahl und Größe dieser Vakuolen, die vorwiegend im apikalen Zellraum angelegt werden, sich aber gelegentlich auch zwischen Kern und Basalmembran finden, nehmen im Verlaufe einer längeren Hungerperiode zu.

Nach dem Genuß von tomatinhaltigen Futter lassen sich solche Vakuolen nicht in der Anzahl wie bei Hungertieren feststellen, doch treten sie bei einzelnen Objekten häufiger auf als bei entsprechenden, normal ernährten Kontrolltieren. Wahrscheinlich ist in diesen Fällen eine Hemmung der Sekretextrusion eingetreten. Bei Kontrolltieren, die nach einer Hungerzeit nur soviel normale Nahrung zu fressen bekamen, wie entsprechende Tiere vom tomatinhaltigen Futter aufnahmen, konnten Vakuolen ebenfalls in etwa gleicher Anzahl gefunden werden. Dies bestätigt die Annahme, daß ihr verstärktes Auftreten nicht auf eine Tomatinwirkung zurückgeht, sondern ein Hungersymptom darstellt, welches sich durch Aufnahme nur geringer Nahrungsmengen nicht beheben läßt.

Die Ausstossung degenerierter Zellen in das Darmlumen verstärkt sich nach Aufnahme von tomatinhaltigem Futter. Diesen Vorgang beobachtet man jedoch auch nach einer 6-stündigen oder längeren Hungerzeit. Er wurde allerlings festgestellt, daß nach dem Genuß von Tomatin die Ausstossung einzelner Zellen durchschnittlich eher eintritt und zahlreicher erfolgt als bei Hungertieren, so daß dieser Vorgang nicht als unmittelbares Hungersymptom angesehen werden kann.

Auflösungserscheinungen im Mitteldarmepithel erfolgen aber in keinem Fall, da die Zahl der abgestossenen Zellen ingesamt gering bleibt.

Vergleichsweise zu diesen Versuchen führten wir Untersuchungen mit der Wildkartoffel *Solanum demissum* Lindl. durch. Wie bereits erwähnt, ist das Hauptalkaloid in *Solanum demissum* ebenfalls Tomatin. Dabei fanden Herkünfte von *Sol. demissum* „Malchow 49", „Reddick 521", „Groß-Lüsewitz 10/57" Verwendung. Während die beiden erstgenannten Klone, unter Berücksichtigung der Kulturmaßnahmen und der sorteneigentümlichen Variabilität, als weitgehend resistent gelten, sind die Pflanzen der Herkunft „Groß-Lüsewitz 10/57" mehr oder weniger anfällig.

Larven und Käfer verhalten sich an *Solanum demissum* in Prinzip ähnlich wie in unseren Veruchen mit tomatinhaltigen Futter. Auch bei histologisch-cytologischen Untersuchungen konnten Abweichungen im Verhalten beider Serien nicht festgestellt werden. Verschiedene, der von CHIN (1950) nach Verfütterung von *Solanum demissum* „Linie 23" im Darmepithel festgestellten Veränderungen konnten in unseren Untersuchungen nicht beobachtet werden.

Bei Larven, die sich an der Herkunft „Groß-Lüsewitz 10/57" entwickelten, ließen sich im Darmlumen ausgestossene Zellen regelmäßig in größerer Anzahl als bei normalen Kontrolltieren finden. Während sich an Pflanzen dieser Herkunft von 100 Versuchslarven mehrere Tiere, wenn auch verzögert, voll entwickelten, gingen von den in gleicher Anzahl an „Malchow 49" und „Reddick 521" gehaltenen Larven alle bereits im L_1- oder L_2-Stadium zugrunde, und zwar unter ähnlichen Begleiterscheinungen wie Larven, die an Tomatin-haltigem Futter in Konzentrationen über 0,5 % gehalten wurden.

Vorliegende Arbeit ist Teil einer von der Mathematisch-Naturwissenschaftlichen Fakultät der Technischen Hochschule Dresden angenommenen Dissertation.

Für die Bereitstellung der Alkaloide sowie für zahlreiche Hinweise danke ich Herrn Dr. K. Schreiber sehr herzlich.

SUMMARY

*INVESTIGATIONS ON THE ACTION OF INSECTICIDES AND PLANT ALKALOIDS ON THE DIGESTIVE TRACT OF LARVAE AND ADULTS OF THE COLORADO BEETLE (*LEPTINOTARSA DECEMLINEATA *SAY)*

The digestive tract of larvae and adults of the Colorado beetle was studied under feeding conditions with different insecticides or alkaloids, moreover the behaviour of the insects was tested on such substances.

Arsenic damages the cuticle of the mid-gut and ultimately causes it to dissolve completely. It was found that an excessive secretion results after ingestion of preparations of DDT and of esters of thiophosphoric acid. A vacuole is formed in the greatly swollen nucleolus.

Physostigmine, Nicotine, Colchicine and Veratrine are very toxic. Nicotine and Physostigmine produce no noticeable histological changes; Veratrine and Colchicine damage the epithelium increasingly. Uptake of Colchicine leads first to abnormal mitosis, later to dissolution of the epithelium. Atropine acts in quantities naturally found in *Atropa belladonna* leaves either by forming tumors or is acutely toxic. Greatly increased doses produce both toxic and tumor forming responses. Tomatine has an exclusive bitter-tasting effect, but as few acute pathogenic changes could be found after uptake of Tomatine as after ingestion of extracts of different sources of *Solanum demissum*.

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ZUM SAUERSTOFFVERBRAUCH VON *MYZUS PERSICAE* (SULZ.) VOR UND NACH AUFNAHME DES BLATTROLLVIRUS

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Der Sauerstoffverbrauch virusfreier *Myzus persicae* (Sulz.) wird mit demjenigen blattrollvirusinfizierter Läuse verglichen. Der O_2-Verbrauch ist bei infizierten Tieren um rund 30% gegenüber virusfreien Kontrolltieren erniedrigt. Auf verschiedenen Wirtspflanzen gehaltene Läuse zeigen keinen signifikanten Unterschied in ihrer Atmungsintensität. Die größte Erniedrigung des O_2-Bedarfs ist etwa 30 Stunden nach Ende des Infektionssaugens (d.h. 38 Stunden nach Beginn der Nahrungsaufnahme) erreicht und steigt dann im Verlauf der folgenden 50 Stunden nicht weiter an. Der Stoffwechsel der Läuse wird offensichtlich durch die Aufnahme des Blattrollvirus gestört.

Die phytopathogenen persistenten Viren, für die eine Vermehrung in ihrem Vektor nachgewiesen ist, benötigen zu ihrer Reproduktion im Insekt die lebende Zelle ebenso wie in der Pflanze. Die pathogenen Veränderungen in der Pflanze sind seit langem bekannt und gut untersucht. Dagegen ist die Frage, ob die persistenten Viren auch einen schädigenden Einfluß auf ihre Vektoren haben, zum großen Teil noch unbeantwortet.

DOBROSCKY (1929, 1931) und BLATTNY (1931), welche Zikaden bzw. Aphiden als Vektoren bestimmter persistenter Viren untersuchten, konnten an diesen keine histologischen Unterschiede gegenüber virusfreien Tieren feststellen. Dagegen wies HARTZELL (1937) gehäuft intrazellulare Körperchen in den Zellen der Speicheldrüse und des Darmtraktus der mit dem Pfirsichgelbsuchtvirus infizierten Zikade *Macropsis trimaculata* Fitch nach. SHUKHOV (1953) fand ebenfalls kristalline Einschlußkörper in den Zellen des Darms, des Fettkörpers und der Speicheldrüsen der Zikade *Calligypona marginata* F., die mit dem Virus des Steckenbleibens des Hafers infiziert war. Diese Körperchen erwiesen sich als infektiös. Schließlich konnten LITTAU & MARAMOROSCH (1956, 1958) beweisen, daß sich das Gelbsuchtvirus der Aster in den Fettkörperzellen des Überträgers in großen Mengen findet und daß es die Zellen pathogen verändert. Auch konnte MARAMOROSCH (1952, 1953) bei Infektionsversuchen mit dem Gelbsuchtvirus der Aster eine erhöhte Sterblichkeit der Zikaden bei der zweiten Passage des Virus und bei hoher Viruskonzentration der Lösung feststellen.

Diese Ergebnisse zeigen, daß das Virus auch im Insekt als Zellparasit aufgefaßt werden kann und eventuell einen nachteiligen Einfluß auf das Insekt als Wirtsorganismus ausübt. In diesem Zusammenhang erhebt sich die Frage, ob die pathogenen Effekte, die durch das Virus im Insekt hervorgerufen werden auf das betreffende Gewebe, etwa den Fettkörper, beschränkt bleiben oder weitere Folgen nach sich ziehen. So wäre denkbar, daß der gesamte Stoffwechsel der Tiere, die ein persistentes Virus aufgenommen haben, beeinflußt wird, besonders bei der Vermehrung des Virus. Da der Gasstoffwechsel sehr leicht Änderungen unterworfen ist, z.B. bei Temperaturwechsel oder Begiftung, lag es nahe, zunächst eine Auswirkung der Virusinfektion auf die Atmung zu untersuchen. Es wurde versucht, diese Frage experimentell zu klären.

Die Versuche wurden mit *Myzus pericae* (Sulz.) und dem Blattrollvirus der Kartoffel durchgeführt. STEGWEE & PONSEN (1958) konnten für dieses Virus eine Vermehrung in der Blattlaus *Myzus persicae* (Sulz.) nachweisen. Aus diesem Grunde schien die Grüne Pfirsichblattlaus für die Versuche am besten geeignet zu sein.

Die über blattrollviruskranke Pflanzen *(Physalis floridana* Rydb.) infizierten Blattläuse und die virusfreien Kontrolltiere, die an *Nicotiana glauca* L. gehalten wurden, stammten aus Gewächshauszuchten, die unter weitgehend konstanten Bedingungen gediehen, sodaß eine gleichbleibende physiologische Kondition der Läuse für alle Versuche vorausgesetzt werden konnte. Die Atmungsmessungen erfolgten mit dem Warburg-Apparat und der KOH-Methode. Für ein Gefäß (15 cm^3) wurden jeweils 100 ungeflügelte Imagines verwendet (Lebendgewicht zusammen etwa 20 mg), die in einen Metallzylinder innerhalb des Reaktionsgefäßes eingekäfigt wurden, um ein Umherlaufen zu verhindern. Alle Versuche wurden bei 25 °C ausgeführt.

Der O_2-Verbrauch virusfreier Läuse wurde an Tieren gemessen, die auf *Nicotiana glauca* gehalten worden waren. Der Mittelwert aus 20 Einzelversuchen mit jeweils 100 Tieren ist aus Tabelle I zu ersehen. Die Abweichung von diesem Mittelwert ist gering, der mittlere Fehler beträgt $\pm$ 0,078, die Standardabweichung (Streuung) $\sigma = 0{,}349$, also 11,5 %. Diese Schwankung ist sicher z. Teil darauf zurückzuführen, daß die Tiere der einzelnen Versuche sich nicht gleichmäßig ruhig verhielten, sondern verschieden starke Bewegungen ausführten. Die erhaltenen Werte des O_2-Verbrauchs fallen in diesselbe Größenordnung, die schon wiederholt für Aphiden festgestellt wurde (TOTH & WOLSKY 1941, MAREK 1959, EHRHARDT & VOß 1960). Die infizierten Läuse hatten etwa 12 Stunden an blattrollviruskranken Pflanzen der Art *Physalis floridana* und danach 30 bis 40 Stunden an *Brassica pekinensis* (Lour.) gesaugt. Die Versuchstiere hatten also die Inkubationszeit des Blattrollvirus (STEGWEE & PONSEN 1958) ohne erneute Virusaufnahme am immunen *Brassica pekinensis* verbracht. Die erhaltenen Werte sind aus Tabelle I zu entnehmen.

TABELLE I

Vergleich des O_2-Verbrauchs virusfreier und infizierter Myzus persicae.
M = Mittelwert. m = mittlerer Fehler. σ = Streuung.

	Anzahl der Versuche	O_2-Verbrauch pro 1 g und pro Stunde	
		M ± m	σ
Virusfreie Virgines	20	3,018 cm^3 ± 0,078	0,349
Mit Blatrollvirus infiz. Virg.	20	1,992 cm^3 ± 0,052	0,234

Vergleicht man die Mittelwerte aus beiden Versuchsserien, so ergibt sich, daß die virushaltigen Läuse einen um 33,9 % niedrigeren Sauerstoffverbrauch als die virusfreien Tiere aufweisen. Dieser Abfall des O_2-Verbrauchs muß in Zusammenhang mit der Infektion durch das Blattrollvirus stehen. Die Vermutung, daß diese Atmungsänderung lediglich durch die verschiedenen Wirtspflanzen bedingt sein könne, wurde durch Versuche widerlegt. In einigen Versuchen wurde der Sauerstoffverbrauch von Tieren bestimmt, die an virusfreien *Physalis*pflanzen und von Tieren die an virusfreien *Brassica*pflanzen gesaugt hatten. Der Mittelwert für den O_2-Bedarf von Läusen von *Physalis* 2,910 cm^3/g/h weicht um 3,5% und der von Läusen an *Brassica* 2,930 cm^3/g/h nur um 2,5 % von dem Mittelwert für den O_2-Verbrauch der Tiere an virusfreien *Nicotiana*pflanzen (3,018 cm^3/g/h) ab. Diese geringen Abweichungen können also nicht für den starken Abfall des Sauerstoffbedarfs der infizierten Läuse verantwortlich sein.

Um festzustellen, wann der Abfall im O_2-Verbrauch nach der Virusaufnahme eintritt, wurden 100 Läuse 8 Stunden auf infizierten *Physalis* belassen. In dieser Zeit konnten sie genügend Virus aufnehmen, um sich zu infizieren. Vorher wurde jedoch ihr O_2-Verbrauch mit 2,95 cm^3/g/h bestimmt. Nach dem achtstündigem Infektionssaugen betrug ihr Sauerstoffverbrauch noch immer 2,90 cm^3/g/h. Die Senkung um 1,7 % liegt wohl innerhalb der Fehlergrenzen der Versuchsmethodik. Nun wurden die Tiere auf *Brassica* übertragen und der O_2-Verbrauch in bestimmten Zeitabständen gemessen. Die Ergebnisse zeigt Tabelle II.

TABELLE II

Abfall des O_2-Verbrauchs mit dem Blattrollvirus infizierter Myzus persicae *in Beziehung zur Zeitdauer nach Aufnahme des Virus*

Zeit in Stunden nach Beendigung des Infektionssaugens	O_2-Verbrauch in cm^3/g/h	Abfall in % gegenüber dem Ausgangswert 2,95
5	2,75	6,8
10	2,67	9,8
20	2,35	20,4
30	2,01	31,9
60	2,05	30,5
80	2,03	31,2

Auch aus diesem Versuch geht hervor, daß nach der Aufnahme des Virus eine signifikante Senkung des O_2-Bedarfs eintritt. Das Maximum des Abfalls ist nach etwa 30 Stunden erreicht; diese Zeitspanne entspricht ungefähr der Inkubationszeit des Blattrollvirus im Insekt. Zur Kontrolle wurde der O_2-Verbrauch virusfreier Tiere, die die gleiche Zeit an *Brassica* gesaugt hatten mit 2,98 cm^3/g/h bestimmt, was dem Ausgangswert entspricht. Die durchgeführten Versuche zeigen, daß nach der Infektion mit dem Blattrollvirus bei *Myzus persicae* eine Senkung der Sauerstoffverbrauchs um etwa 30 % eintritt. Dieser Abfall setzt erst nach Ende des Infektionssaugens ein, erhöht sich während der Inkubationszeit und erreicht am Ende derselben ein Maximum, um danach annähernd konstant zu bleiben. Diese Beziehung zur Inkubationszeit ist wohl durch die Vermehrung des Virus in der Laus zu erklären. Ob dieser Abfall im Sauerstoffverbrauch die Folge eines pathogen gestörten Stoffwechsels ist, bleibt ungewiß.

Vorliegende Arbeit wurde während eines Gastaufenthaltes am Institut für Entomologie der Landwirtschaftlichen Hochschule zu Wageningen/Niederlande ausgeführt. Dem Direktor des Entomol. Instituts, Herrn Professor Dr. J. de Wilde bin ich für die gastliche Aufnahme, die Anregung zu diesem Problem und alle freundliche Unterstützung zu großem Dank verpflichtet. Herr Professor Dr. T. H. Thung, dem Direktor des Instituts für Virologie, sei für die freundliche Überlassung eines Arbeitsplatzes gedankt. Herrn M. B. Ponsen danke ich für seine bereitwillige Unterstützung und Hilfe.

SUMMARY

ON THE OXYGEN CONSUMPTION OF MYZUS PERSICAE *(SULZ.) BEFORE AND AFTER THE UPTAKE OF POTATO LEAFROLL VIRUS*

The intensity of respiration of virus-free aphids was compared with that of the same insects infected with potato leafroll virus. The former showed an average oxygen-consumption of 3.018 cm³/gm/hour, and the latter 1.992 cm³/gm/hour; which indicates a reduction of 33.9% in the oxygen-uptake in the infected animals. This reduction is associated with the infection and the multiplication of the virus in the insect. The oxygen-consumption was the same before the beginning and at the end of the infective suction period, falling afterwards over a period of 30 hours by about 30% of the first reading, and then remaining almost constant. This indicates a correlation between this variation in the oxygen-consumption and the multiplication of the virus in the aphid.

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EFFECT OF THE MUTILATION OF WINGS ON THE REPRODUCTION OF THE BEAN APHID *APHIS FABAE* SCOP.

BY

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Mutilation of the wings by pinching and twisting them immediately after the aphid emerged hastened its reproduction. The hastening was more striking among individuals so treated which were kept in light than those kept in dark. Reproduction was also hastened, but to a slighter degree, among individuals with naturally deformed wings.

It has been shown by earlier workers that bodily exercises hastened reproduction. The facts revealed in the present paper suggest that the nervous changes resulting from bodily exercise are more important than the actual exercises themselves.

B. Johnson (1955) showed that exercise, particularly flight, and exposure to light initiated settling down, probing and reproduction of alatae of the bean aphid *Aphis fabae* Scop. I wished to find whether some other stimuli would have the same effects; experiments which included chilling and heating gave no positive results.

Quite by accident, it was found that mutilation of the wings immediately after the aphid emerged produced the desired results, namely, the hastening of reproduction. De Fluiter (1949) used the method of clipping off the wings to keep the insect on its host plant. He did not, however, study the effects of this procedure on the biology of the insect beyond stating that no adverse effect was observed.

The procedure employed in the present study was rather simple, namely, pinching and twitching the wings together with a pair of forceps. Since the wings were not yet hardened, they stuck together and stayed so throughout the life of the aphid. About 20 aphids so treated were kept individually on a young bean plant under a screened lamp chimney under light. A similar number was kept in the dark. The aphids in the latter group were confined in individual small cages as described by Kennedy (1950). The number of young produced by each of the aphids was checked 24 hours and 48 hours after their emergence. Some additional observations were also made in order to determine the time when reproduction began. Comparable records were also kept on aphids with normal wings. In the aphid culture there were individuals the wings of which were not fully expanded. The appearance of these wings was very similar to that of the mutilated wings. Records were kept also on the reproduction of these aphids with naturally deformed wings.

Results on the reproduction of the individuals of the three groups and under two lighting conditions are shown in Fig. 1. The following points are shown.

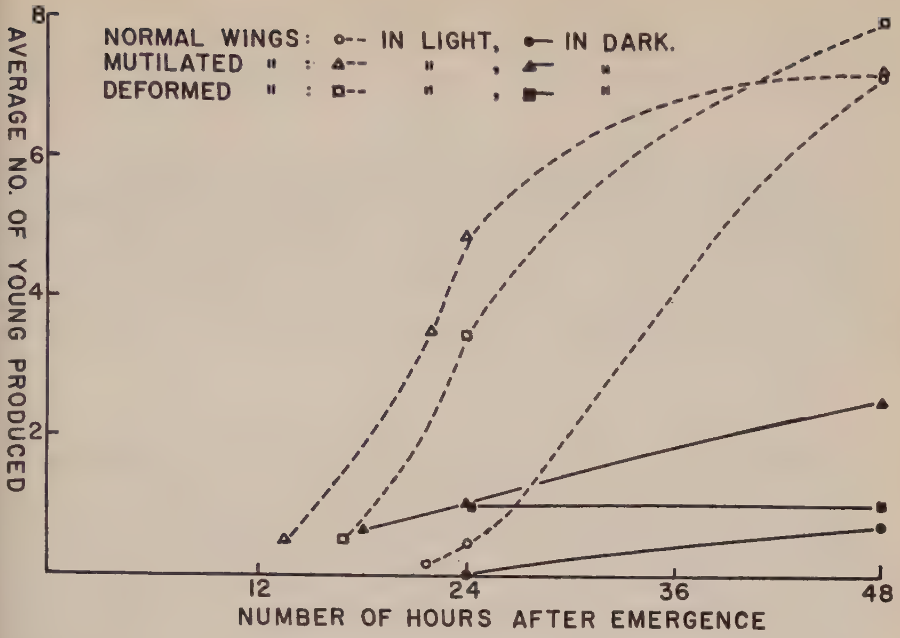

Figure 1. Total number of young produced by aphids of three types of wings, under two lighting conditions, and up to various ages. (Numbers given are averages from 15—20 aphids)

1. The aphids kept in the light started reproduction earlier and produced more young than those kept in the dark.
2. With the same light exposure, aphids with mutilated wings started reproduction earlier and produced more young than did those with normal wings, and the aphids with deformed wings were intermediate in both the rate of reproduction and the number of young produced.
3. The greatest contrast in the rate of reproduction was shown therefore between the mutilated individuals in the light and the normal individuals in the dark. In the former case reproduction started at 13 hours after emergence and in the latter, at more than 36 hours.

It was observed that upon reaching flight readiness the aphids with normal wings and in the light started to walk on the leaf and fluttered about near the muslin screen of the chimney for 2 to 3 hours, then they visited the leaf again at intervals. It is conceivable that under such conditions the aphids had carried on a considerable amount of exercise. In the dark, however, they usually do not take up much activity, and the confinement in the small cage further ensured that the aphids did not flutter about. It was further observed that aphids with mutilated or deformed wings did not fly and did only a limited amount of walking even in the light.

It was mentioned above that flight as a normal activity of the winged aphids hastened reproduction. Yet, mutilation of the very organ for flight also hastened reproduction. This result appeared rather paradoxical since these aphids never flew and hardly even walked about. This perplexing finding, however, may be explained by the assumption offered by B. Johnson (1955) that the relation between the locomotor and settling response was mainly through the nervous centre.

The work was done during the author's tenure as a Guggenheim Fellow. The author is indebted to Professor V. B. Wigglesworth, Cambridge University, in whose laboratories this work was carried out. Grateful acknowledgment is due also to Dr. J. S. Kennedy for making available fine facilities and for stimulating discussions.

Dr. Robert J. Wolffs' kindness in reading and interpreting the paper by De Fluiter is gratefully acknowledged.

ZUSAMMENFASSUNG

DIE WIRKUNG DER FLUGELVERSTÜMMELUNG AUF DIE FORTPFLANZUNG DER SCHWARZEN BOHNENLAUS, APHIS FABAE *SCOP.*

Verstümmelung der Flügel durch Zusammenpressen und -drehen gleich nach der Häutung beschleunigte die Fortpflanzung der Blattläuse. Diese Beschleunigung war unter im Licht gehaltenen Tieren auffallender als unter solchen, die in der Dunkelheit gehalten wurden. Die Fortpflanzung ging auch bei solchen Blattläusen — wenn auch im geringeren Grade — schneller vor sich, die ohne künstlichen Eingriff schon mit verstümmelten Flügeln schlüpften.

Von früheren Autoren ist gezeigt worden, daß auch körperliche Betätigung die Fortpflanzung beschleunigte. Die Befunde der vorliegenden Arbeit weisen nun darauf hin, daß die durch körperliche Bewegung verursachte Nerventätigkeit wichtiger ist als die Ausübung selbst.

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STUDIES IN DIURNAL RHYTHMS – X. SYNCHRONISATION OF THE ENDOGENOUS CHRONOMETER IN *BLABERUS GIGANTEUS* (L.) (DICTYOPTERA: BLATTARIA) AND IN *GRYLLUS CAMPESTRIS* L. (ORTHOPTERA: GRYLLIDAE)

BY

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The cockroach, *B. giganteus*, is nocturnal, showing an endogenous, temperature-independent rhythm of activity and rest. This rhythm is delayed by constant illumination and activity is suppressed by bright light: the rhythm is not influenced by constant darkness. Unlike that of the field cricket, *G. campestris*, the rhythm of *B. giganteus* is not reset or re-established by 3-hour periods of light or darkness, nor by return to higher temperatures after a period at 5° C. The rhythm of *G. campestris* is delayed by dark and not affected by constant light in which it is not reset, although is may be shifted by a 3-hour period of darkness. The suggestion is made that the rhythm of the cockroach reflects a greater degree of central control than that of the cricket.

In a previous paper in this series (CLOUDSLEY-THOMPSON, 1958) the 24-hour rhythm of activity and rest was investigated in a day-active cricket, *Gryllus campestris* L. The present study comprises the results of comparable but more extensive experiments carried out on a night-active insect, the giant South American cockroach, *Blaberus giganteus* (L.), with further observations on *G. campestris.*

The effects of continuous light and darkness on persistent, or endogenous rhythms have been reviewed by ASCHOFF (1958) and HARKER (1958). Aschoff's conclusions, based largely upon vertebrate studies, can be summarised as follows: when night-active animals are placed in constant light, their rhythms tend to be delayed, whilst those of day-active forms may be accelerated: the converse occurs in darkness. Synchronisation with diurnal environmental changes cannot be achieved both at dawn and at dusk, as the time of each of these is altering. The significant factor is dusk in the case of nocturnal forms, dawn in that of day-active animals. A similar conclusion has been reached in the case of terrestrial arthropods (CLOUDSLEY-THOMPSON, 1959a, b) but here the evidence is more scanty. Nevertheless, if the above generalisations can be shown to be valid, they indicate a way in which diurnal rhythms may be shifted as the days lengthen, or draw in according to the season.

MATERIAL AND METHODS

Experiments were undertaken from November 1958 to December 1959, on cockroaches, *Blaberus giganteus*, bred in the laboratory. My thanks are due to

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Dr. J. D. Carthy for the gift of a culture of these animals. The crickets were obtained by me in France during August, 1959. Both species were maintained in the laboratory in natural daylight and darkness at room temperature, and were fed on rat cake, bread, apple, lettuce etc. The influence of different regimes of lighting and temperature upon their locomotory activity was measured as before by means of aktograph apparatus (Cloudsley-Thompson, 1958).

RESULTS

Examples of the results obtained with *Blaberus giganteus* are given in Figs 1-3. From examination of these and others unpublished, the following facts can be adduced:

1. *B.giganteus* is a nocturnal animal in all stages of its development (Fig. 1, top row).
2. The rhythm persists and tends sometimes to be accentuated under constant conditions (Figs 1-3).
3. The phase of the rhythm is temperature-independent within limits, but activity is usually greater at higher temperatures (Fig. 3, third and fourth rows).

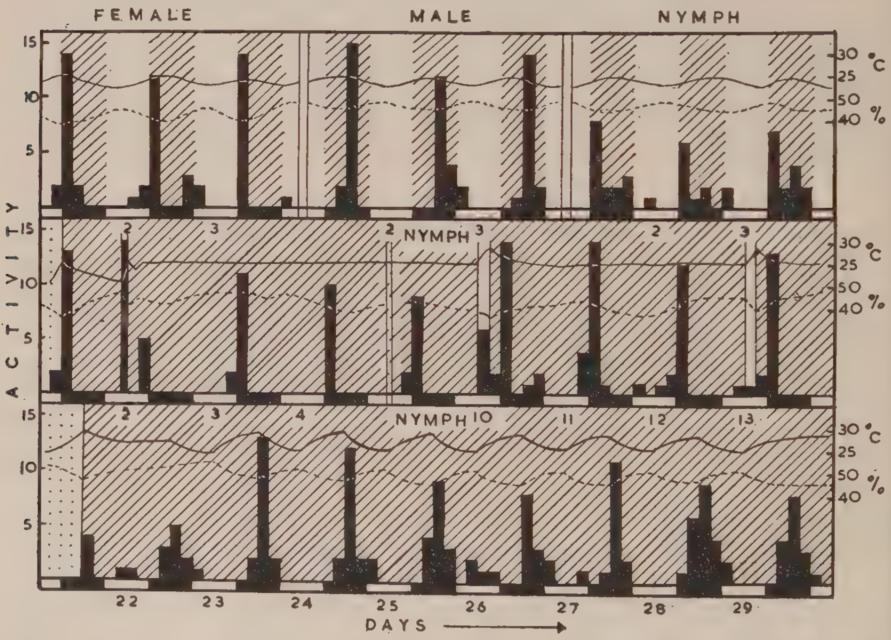

Fig. 1. Analysis of aktograph records showing periodicity of movement and rest in *Blaberus giganteus*. Ordinates, left: activity; right; temperature (solid line) and relative humidity (broken line). Black stripes: 12-hr. periods from 18.00 tot 06.00 hr; oblique lined areas: periods of darkness; dotted areas: periods with light. Numbers indicate how many days each insect had been in the apparatus at the time that the record was made. Top row: activity rhythm of female, male and nymph under room conditions. Centre row: persistence of the rhythm (synchronised by change from light to darkness) in constant darkness and the effect of 3-hr. periods of light. The record for days 5—9 has been omitted as it was similar to that for days 3—4. Bottom row: persistence of the rhythm in constant darkness with fluctuating temperature and relative humidity.

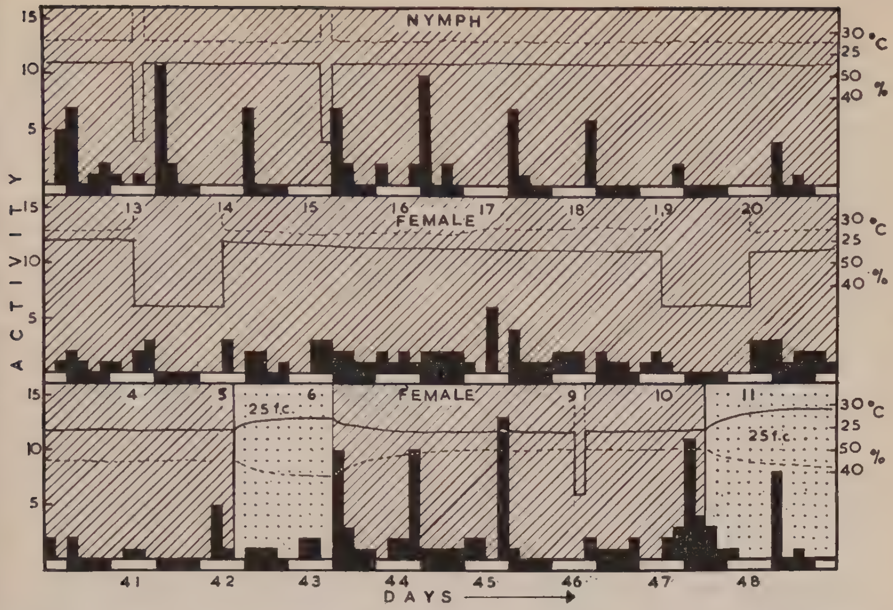

Fig. 2. Analysis of aktograph records showing periodicity of movement and rest in *Blaberus giganteus*. Conventions as in Fig. 1. Top row: effect of 3-hr. at low temperature on the diurnal rhythm. Centre row: effect of 24 hr. at low temperature on the activity of an insect which had lost its rhythm after a long period in constant conditions. Bottom row: effect of periods of light and of low temperature on the rhythm of another arhythmic insect.

4. The rhythm is *delayed* in constant light (25 ft. candles) (Fig. 3, first and second rows).
5. Activity is suppressed by bright light (125 ft. candles) (Fig. 3, first, and second rows).
6. The rhythm is neither accelerated nor delayed in constant darkness (Figs 1-3).
7. The rhythm is not re-set or re-established by a 3-hour flash of light (as occurs in the cricket, *Gryllus campestris* according to CLOUDSLEY-THOMPSON, 1958) although activity may be stimulated by the light (Fig. 1, centre row).
8. Conversely, the rhythm is not re-set by a 3-hour period of darkness after a period in constant light (25 ft. candles) although activity is stimulated by the darkness (Fig. 3, bottom row).
9. The rhythm is occasionally re-set by exposure to light after a long period of darkness (Fig. 3, bottom row).
10. The rhythm is re-set by exposure to darkness after a long period of light (Fig. 1, centre and bottom rows; Fig. 2, bottom row) or by reduction in light intensity to 25 ft. candles after activity has been suppressed by higher light intensity (125 ft. candles) (Fig. 3, second row).
11. The rhythm is not re-set by return to warm conditions after a period at low temperature (5-10° C) as occurs in *G. campestris* (CLOUDSLEY-THOMPSON 1958) (Fig. 2).

Aktograph experiments on the cricket, *Gryllus campestris*, elicited two points not established in the earlier paper on this species (CLOUDSLEY-THOMPSON,

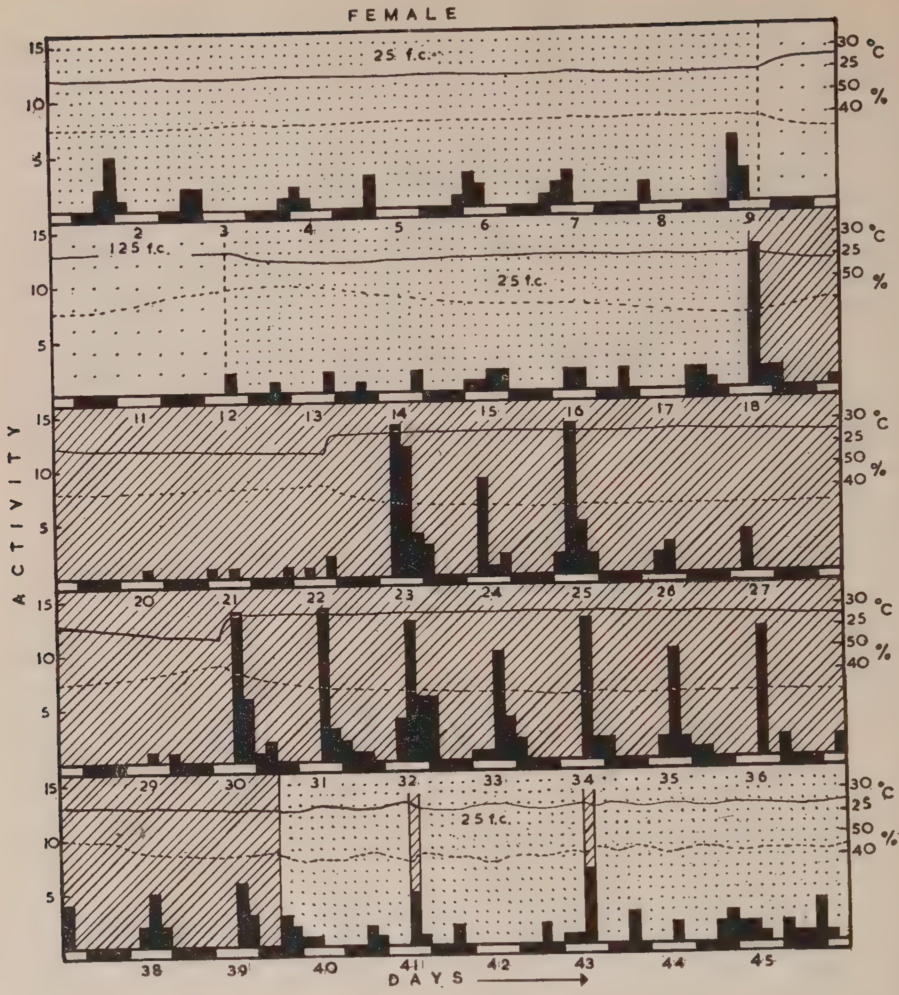

Fig. 3. Analysis of aktograph records showing periodicity of movement and rest in *Blaberus giganteus*. Conventions as in Fig. 1. First and second rows: effect of constant light (25 and 125 ft. candles) on the rhythm. Third and fourth rows: effect of higher and lower temperatures on the rhythm in continual darkness. Bottom row: effect of the change from dark to light and of 3-hr. periods of darkness on the rhythm in light (25 ft. candles).

1958). In the figures previously published it can be seen that the rhythm tends to be retarded by constant dark. From Fig. 4 (below) it can also be observed that, in addition:

1. The rhythm of *G. campestris* is not accelerated by constant light and does not persist in light to the same extent as it does in darkness.
2. Whereas the rhythm in darkness has previously been shown to be re-set by 3 hours' exposure to light, the rhythm in constant light is not re-set by a 3-hour period of darkness, although subsequent peaks may perhaps be shifted by this amount of time.

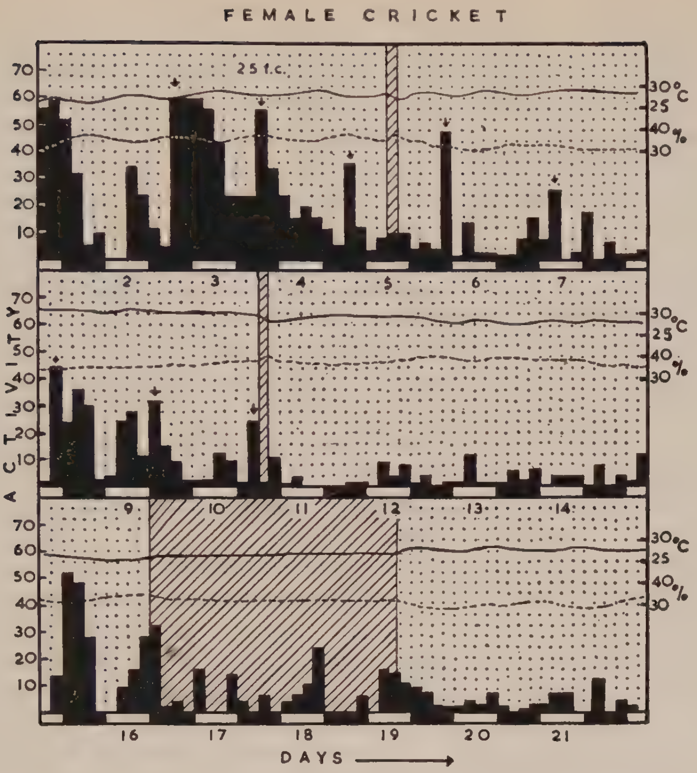

Fig. 4. Analysis of aktograph records showing periodicity of movement and rest in *Gryllus campestris.* Conventions as in Fig. 1. Top and centre rows: effect of 3 hr. periods of darkness on the rhythm in continual light (25 ft. candles). The small arrows indicate main peaks of activity. Bottom row: effects on the rhythm of changes from light to darkness and vice versa.

DISCUSSION

Comparison of the endogenous rhythms of locomotory activity in the nocturnal cockroach, *Blaberus giganteus,* and the day-active cricket, *Gryllus campestris,* lends support to the views outlined in the introductory paragraphs. The rhythm of the cockroach is delayed by constant light, that of the cricket by darkness. In neither insect does the rhythm appear to be accelerated. The frequency of the periodicity remains almost exactly at 24 hours for the cockroach in darkness and the cricket in light, these being the conditions in which the respective species are normally active under natural conditions.

An important difference in the mechanism of the periodicity in the two insects is suggested by the fact that in the cricket the internal "clock" can easily be re-set by brief exposure to light, or by return to higher temperatures after a period at 5°C, whereas in the cockroach it is more stable. HARKER (1956) has shown that in the cockroach, *Periplaneta americana* L., the activity rhythm is controlled by a hormone secreted by the sub-oesophageal ganglion and it is highly probable that a similar mechanism obtains in *B. giganteus.* In *G. campestris,* on other hand, it has been suggested that there may be a number of internal "clocks" each possessing an inherited 24-hour periodicity and normally synchronised by the change from darkness to light. After a while in constant conditions these tend to get out of phase with one another so that the rhythm gradually disappears. They can be re-set again by a single exposure to light, or by a return to warmth after a period at low temperature (CLOUDSLEY-THOMPSON, 1958). The differences observed between the periodicities of the cockroach and the cricket may reflect a greater degree of central control in the former, in which it is probable that the sub-oesophageal ganglion acts as a "master-clock".

JANDA & MRCIAK (1957) have shown that the locomotory rhythm of *P. americana* is paralleled by a rhythm of oxygen consumption and similar results have been obtained with the American grasshopper, *Romalea microptera* (Beauvois) by FINGERMAN, LAGO & LOWE (1958). Grasshoppers whose sub-oesophageal ganglia had been removed showed no rhythm but implanted ganglia failed to induce a rhythm in individuals whose sub-oesophageal ganglia had been removed. Clearly the mechanism of rhythmic control must vary in different species and it may even be that a centre of rhythmicity operates through the suboesophageal ganglion which, in turn, stimulates locomotory activity.

BÜNNING (1959) has suggested that the diurnal periodicity of *P. americana* is based on oscillations of relaxation and that the normal 24-hour cycle of daylight and darkness regulates these oscillations in such a way that a phase of tension begins in the second half of the light period and attains a maximum in the first part of the dark period. It is not clear, however, whether activity occurs at the time of hormonic secretion, or if the hormone is inhibitory. According to HARKER (1956) there is some evidence for it being inhibitory, at least in high concentration, under certain conditions.

ZUSAMMENFASSUNG

STUDIEN ÜBER DIURNALE RHYTHMIK. X. DIE SYNCHRONISATION DES ENDOGENEN ZEITMESSERS BEI BLABERUS GIGANTEUS *(L.) (DICTYOPTERA: BLATTARIA) UND BEI* GRYLLUS CAMPESTRIS *L. (ORTHOPTERA: GRYLLIDAE)*

Bei der Schabe *Blaberus giganteus* wurde die tägliche Rhythmik von Aktivität und Ruhe mittels eines Aktographen untersucht. Das Insekt ist in allen Entwicklungsstadien nächtlich und zeigt eine endogene Periodizität, die innerhalb gewisser Grenzen temperaturunabhängig ist: es ist bei höheren Temperaturen gewöhnlich lebhafter. Der Rhythmus wird durch konstante Bedeuchtung (25 f.c.) verzögert und die Aktivität bei hellem Licht (125 f.c.) unterdrückt: sie wird von konstanter Dunkelheit nicht beeinflußt. Der Rhythmus wird weder durch 3-stündige Lichtperioden, noch — wenn das Insekt in konstanter Beleuchtung lebt — durch 3-stündige Dunkelperioden verstellt. Er wird nach einem Aufenthalt bei 5° oder 10° C auch nicht durch Rückkehr zu höheren Temperaturen verstellt. Er kann jedoch durch Dunkelheit nach einer Lichtperiode (oder durch eine plötzliche Reduktion der Lichtintensität) und gelegentlich durch Licht nach einem langen Dunkelaufenthalt synchronisiert werden.

Im Gegensatz dazu zeigt die tagaktive Grille, *Gryllus campestris*, einen endogenen Rhythmus, der durch Dunkelheit verzögert und von konstanter Beleuchtung nicht beeinflußt wird. Er wird darin nicht wiederhergestellt, obwohl er vielleicht durch eine 3-stündige Dunkelperiode verschoben werden kann. Es wird wahrscheinlich gemacht, daß der Rhythmus der Schabe einen größeren Grad zentraler Kontrolle als der der Grille darstellt.

Diese Ergebnisse bedeuten, daß die Art der Synchronisation der Rhythmen der Insekten mit den jahreszeitlichen Änderungen der Tageslängen ähnlich der bei Säugern und Vögeln bereits bekannten verläuft.

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UNTERSUCHUNGEN UBER DEN AMINOSÄUREGEHALT DES SPEICHELDRÜSENSEKRETES DER REBLAUS (*VITEUS [PHYLLOXERA] VITIFOLII* SHIMER), HOMOPTERA

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Im Reblausspeichel wurden papierchromatographisch fünf Aminosäuren, zwei Amide und wahrscheinlich ein Peptid nachgewiesen. Herkunft und Bedeutung dieser Verbindungen im Speichel werden diskutiert.

Anders (1957, 1958) stellte fest, daß der Reblausspeichel in relativ hoher Konzentration (mindestens 10 %) freie Aminosäuren enthält. Mit Hilfe der eindimensionalen, aufsteigenden Papierchromatographie und verschiedenen Nachweisreagenzien konnte er die Aminosäuren Lysin, Histidin, Tryptophan und wahrscheinlich Glutaminsäure und Valin identifizieren. Diese Aminosäuren werden als die gallenerregenden Stoffe des Reblausspeichels betrachtet und daraus, besonders für Tryptophan, weitreichende Schlußfolgerungen gezogen (Anders, 1959). Das Problem der Gallbildung erschien durch diese Arbeiten in einem neuen Licht.

Eine Überprüfung dieser Befunde wurde jedoch erforderlich, als Vorversuche mit Reblausspeichel keine Bestätigung der Anders'schen Analysenergebnisse erbrachten.

Anders sammelte den Speichel, indem er die Rebläuse in Rückenlage festklebte und die an der Stechborstenspitze austretenden Sekrettröpfchen absammelte. Bei den eigenen Untersuchungen wurde zuerst in gleicher Weise verfahren, doch zeigte sich bald, daß diese Methode zu langwierig ist, um brauchbare Ausgangskonzentrationen für eine klare Identifizierung der Aminosäuren zu ermöglichen, denn bei weitem nicht alle Rebläuse sondern bereitwillig ihren Speichel ab. Auf den hohen Arbeitsaufwand und die geringen Speichelmengen, die damit gewonnen werden können, weist Anders (1958) selbst hin.

Bei den durchgeführten Serienuntersuchungen wurde deswegen der Speichel erwachsener Blattrebläuse mit Hilfe einer wirkungsvolleren Methode gewonnen (erwachsene Rebläuse können für die Untersuchungen benutzt werden, da Vorversuche ergaben, daß sie wie Jungläuse zur Gallbildung befähigt sind). Die Rebläuse wurden innerhalb eines Glasringes auf schwach angefeuchtetes Chromatographie-papier gesetzt und während der Versuchsdauer nicht gestört. Kurze Zeit nach dem Ansatz begannen die Läuse in das Papier einzustechen. Dabei gibt das Insekt reichlich Speichel in das Papier ab (Kloft, 1956, Kloft & Ehrhardt, 1959). Die Reblaus sticht besonders dann in das Papier ein, wenn von der Unterseite her durch eine Glasplatte Tageslicht auf das Chromatographie-papier fällt. Nach 6 - 8 Stunden, bei einigen Ansätzen auch nach 24 Stunden, wurden die Rebläuse, die z. T. noch mit tief angestochenen Stechborsten am Papier festhingen, entfernt. Mit zunehmender Versuchsdauer nimmt die Einstichhäufigkeit ab, so daß es zwecklos ist, die Tiere noch länger auf dem Papier zu lassen.

Damit wurde der Reblausspeichel zwar nicht nach der gleichen Methode wie von Anders angereichert, doch kann dies wohl kaum für die stark abweichenden Analysenergebnisse verantwortlich gemacht werden; es sei denn, daß die von Anders gesammelten Flüssigkeitströpfchen an der Stechborstenspitze außer Speichel noch Inhaltsstoffe des Darmes enthalten, denn ein Rücktransport dieser Stoffe, (Erbrechen) ist bei der unnatürlichen Rückenlage der Rebläuse nicht ausgeschlossen (KLOFT, 1956). In diesem Fall hätte Anders keinen reinen Speichel untersucht und der Wert der Analysenergebnisse für die Klärung der Wechselbeziehungen zwischen Wirt und Parasit wäre in Frage gestellt, denn in Verbindung mit der normalen Nahrungsaufnahme wurde eine Regurgitation von Darminhalt bei Aphiden, insbesondere bei der Reblaus, meines Wissens noch nicht beobachtet. Nach dem derzeitigen Wissen über diese Vorgänge übt bei Aphidenbefall das reine Speicheldrüsensekret den physiologischen Reiz auf den Wirt aus.

Mit der bei den eigenen Versuchen angewandten Methode konnten relativ schnell größere Speichelmengen gewonnen und der papierchromatographischen Analyse zugeführt werden, unserer Ansicht nach eine unerlässliche Voraussetzung für eine eindeutige Identifizierung der Aminosäuren. Für jedes zweidimensionale Chromatogramm wurde der Speichel von ca. 700 erwachsenen Blattläusen (8 Ansätze mit je 80 - 90 Individuen) gesammelt. Die Reblaus eignet sich für diese Methode der Speichelgewinnung besonders, weil im Verlaufe der Entwicklung zum erwachsenen Tier die Afteröffnung verschlossen wird. Bei erwachsenen Tieren entfällt damit die störende Exkretion. Die bei der Eiablage mit ausgeschiedenen Exkrete können vernachlässigt werden. Entweder befinden sie sich unterhalb des Chorions (GERSCH, 1953) oder sitzen als Polkappen dem Ei auf (HALLE, 1959). In letzterem Fall ist jedoch die Flüssigkeitsmenge und die möglicherweise darin enthaltene Aminosäuremenge so gering, daß eine Beeinflussung der Speicheluntersuchungen ausscheidet. Werden z.B. ca. 1000 Reblauseier eine Stunde in Aqua dest. gewaschen und die Waschflüssigkeit eingeengt und chromatographiert, so lassen sich keine ninhydrin-positiven Substanzen nachweisen. Bei den einzelnen Ansätzen zur Speichelgewinnung werden jedoch bei entsprechender Auswahl der Versuchstiere während der Versuchsdauer im Höchstfall 200-300 Eier abgelegt.

Besonders zu beachten ist allerdings bei dieser Methode, daß die erwachsenen Blattläuse unverletzt aus den Gallen herauspräpariert und auf das Papier übertragen werden. Durch austretende Lymphe (sie enthält freie Aminosäuren) würde eine Speicheluntersuchung unmöglich gemacht. Geschieht es trotzdem einmal, so müssen die schwach gelb gefärbten Stellen aus dem Papier ausgeschnitten werden.

Weiterhin ist zu bedenken, daß wohl reines Speicheldrüsensekret in das Papier abgegeben wird, doch ob es genau die gleiche chemische Zusammensetzung aufweist wie bei dem Einstich in Pflanzengewebe, ist fraglich. Auf diese Schwierigkeit hat schon KLOFT (1956) hingewiesen, doch ist sie allen bekannten Methoden zur Speichelgewinnung von Aphiden eigen und vorläufig nicht zu umgehen.

Für die weitere Verarbeitung wurde der Reblausspeichel aus dem Chromatographie-Papier der einzelnen Ansätze mit 70 %igem Alkohol eluiert, das Eluat auf 0,5 ml eingeengt und quantitativ auf ein zweidimensionales Chromatogramm aufgetragen (eindimensionale Chromatogramme brachten nicht die gewünschte klare Auftrennung). Die erste Phase lief in Butanol : Ameisensäure : Wasser = 75 : 15 : 10 und die zweite Phase in gereinigtem 80 %-igem Phenol. Bei beiden Phasen wurde aufsteigend chromatographiert. Als Reagenz diente bei den Serienuntersuchungen 0,2 %-iges Ninhydrin in wassergesättigtem Butanol. Andere Lösungsmittelgemische kamen ebenfalls zur Anwendung, doch trennte Phenol in der 2. Phase die Aminosäuren des Speichels am günstigsten. Durch die klare Trennung und meist eindeutige Lage der ninhydrin-positiven Substanzen im zweidimensionalen Chromatogramm bereitete die Identifizierung mit Reinsubstanzen keine Schwierigkeiten.

Die folgende Zusammenstellung (Tabelle I) der im Reblausspeichel gefundenen Aminosäuren und Amide enthält zugleich Angaben über die vermutliche Konzentration. Auf eine genaue quantitative Bestimmung mußte bei diesen Untersuchungen noch verzichtet werden. Bei der halbquantitativen Bewertung wurde die Farbintensität und die Größe der Flecken auf dem Papier abgeschätzt und vier willkürlich gewählten Größenklassen (+, ++, +++, ++++) zugeordnet, wie es z.B. ROHRINGER (1957) getan hat. Die Angabe „+" bedeutet dabei die geringste Konzentration. Mit Hilfe von Reinsubstanzen auf Vergleichschromatogrammen konnten diesen Klassen die aus Spalte „b" der Tabelle I ersichtlichen Aminosäurekonzentrationen zugeordnet werden.

TABELLE I

Zusammenstellung der Aminosäuren und Amide des Reblausspeichels

	a	b
Asparagin	+++	10—12 γ
Glutamin	+++	8—10 γ
Glutaminsäure	+	1— 3 γ
Alanin	++	4— 6 γ
Serin	+	1— 3 γ
Glycin	+	1— 3 γ
Arginin	+	1— 3 γ
Nicht identifizierte Substanz (s. Abb. 1)	++	4— 6 γ (?)

a: Größenklasse der Flecken; b. Konzentration

Die Zahl der im Speichel gefundenen Aminosäuren, Amide und Peptide (?) ist überraschend groß und dürfte sich bei weiterer Konzentrationserhöhung des Ausgangsmaterials noch vergrößern.

So wurden z.B. auf einigen Chromatogrammen außer den in der Tabelle I aufgeführten Aminosäuren noch Asparaginsäure, Threonin, Tyrosin und Valin festgestellt (s. schraffierte Orte der Abb. 1). Gesichert ist damit jedoch das Vorkommen dieser Aminosäuren im Speichel noch nicht.

Den größten Anteil am Gehalt an ninhydrinpositiven Substanzen haben zweifellos die beiden Amide Asparagin und Glutamin. Es überrascht, daß Asparagin von Anders nicht gefunden wurde, obwohl es durch seine gelbbraune Farbreaktion mit Ninhydrin unverkennbar ist. Möglicherweise trat im eindimensionalen Chromatogramm die gelbbraune Farbe des Asparagins durch überlagerung mit anderen Aminosäuren nicht deutlich in Erscheinung. übereinstimmung mit den Befunden von Anders besteht nur im Falle der Glutaminsäure. Seine Angaben, daß Lysin, Histidin und Tryptophan im Reblausspeichel auftreten, konnten nicht bestätigt werden.

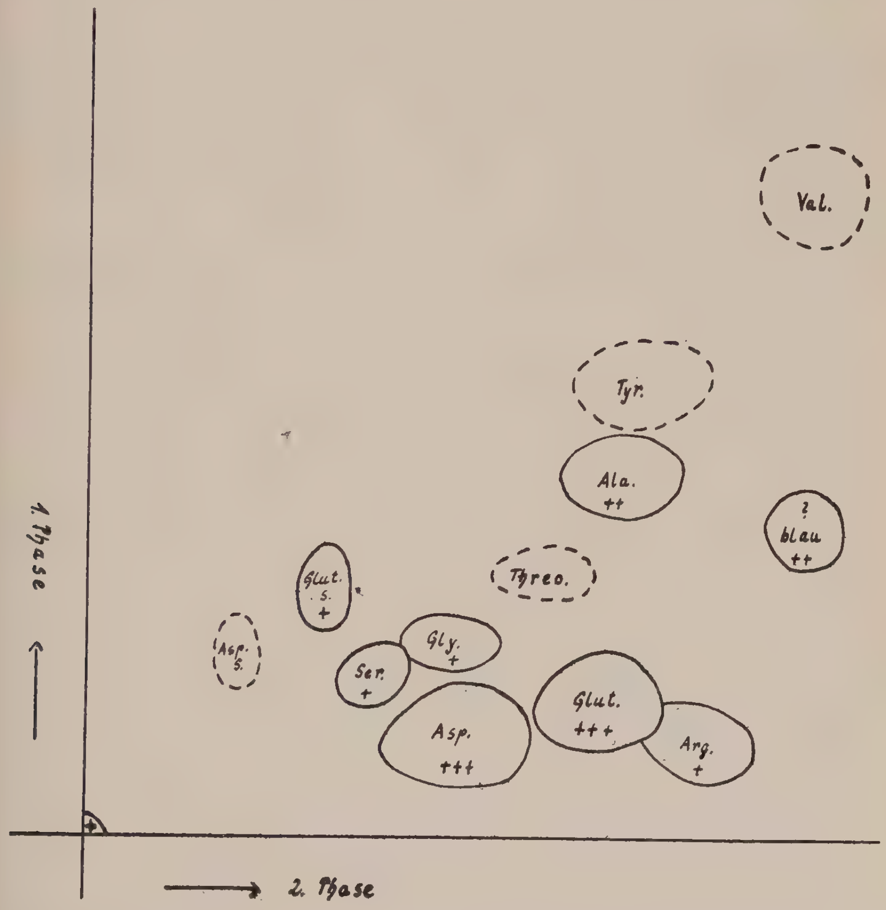

Abb. 1: Aminosäuren und Amide des Reblausspeichels. 1. Phase: Butanol — Ameisensäure — Wasser = 75 : 15 : 10. 2. Phase: 80%-iges Phenol. Weitere Erklärungen im Text.

Da Tryptophan und Histidin mit Ninhydrin verhältnismäßig schwache Farbreaktionen geben, wurde die Abwesenheit dieser Aminosäuren im Reblausspeichel zusätzlich durch spezifische Nachweisreaktionen überprüft. Zu diesem Zweck war es notwendig, in der 2. Phase der Chromatogramme nicht Phenol, sondern das Gemisch Butanol : Methanol : Wasser = 4 : 5 : 1 zu verwenden. Als Reagenzien dienten p.-Dimethylamino-benzaldehyd (Ehrlich's Reagens) zum Nachweis von Tryptophan und diazotierte Sulfanilsäure zum Nachweis von Histidin (LINSKENS, 1955). In beiden Fällen wurden nicht einmal Spuren dieser Aminosäuren bemerkt, so daß deren Abwesenheit im Speichel mit Sicherheit angenommen werden darf. Anders schreibt, daß Tryptophan außer mit Ninhydrin noch in anderen Versuchen nach WINKLER & PETERSEN (1935) bestimmt wurde. Auf diesen Befund soll jedoch im Rahmen dieser Arbeit nicht näher eingegangen werden, weil mit dem von Winkler & Petersen benutzten Reagenz (Glyoxylsäure in Gegenwart von Kupfersulfat und konzentrierter Schwefelsäure) außer Tryptophan auch noch β-Indolylessigsäure farbige Verbindungen ergibt. Damit wird das Problem des Wuchsstoffgehaltes von Insektenspeichel berührt — ein Problem, zu dem noch eingehende Untersuchungen durchgeführt werden müssen. Zwar wies ANDERS (1957) mit der Methode nach GORDON & WEBER (1951) — einer Variation der Salkowski-Reaktion — im Reblausspeichel keine β-Indolylessigsäure nach, doch widersprechen diesem Befund die Angaben von HOPP (1958), der mit der gleichen Reaktion und außerdem mit der fluorometrischen Methode nach Hamence β-Indolylessigsäure im Reblausspeichel feststellte.

Auf spezifische Nachweisreaktionen für Lysin wurde verzichtet. Lysin gibt mit Ninhydrin eine kräftige violette Farbreaktion, so daß es im zweidimensionalen Chromatogramm kaum übersehen werden kann. Der mit Gemischen von Reinsubstanzen ermittelte Ort von Lysin auf den Chromatogrammen zeigte jedoch bei allen Versuchsreihen keine Farbreaktion. Anders schreibt, daß Lysin bereits in Vorversuchen an der nach Besprühen eines Chromatogramms mit Folin-Reagens auftretenden charakteristischen Färbung (dunkeltaubenblau) erkannt wurde. Folin-Reagens auf Aminosäurestickstoff (1,2-Naphthochinon-4-sulfonsaures Natrium) wurde auch bei den eigenen Versuchen angewandt (nicht als spezifisches Reagens für Lysin), doch wurden damit keine überzeugenden Ergebnisse gewonnen. Nach MÜTING (1952) reagiert z.B. Alanin „taubenblau", Glycin „helltaubenblau", Ornithin „himmelblau", einige Aminosäuren „blaugrau" oder „graublau" und dazu Lysin „dunkeltaubenblau". Daß bei diesen Farbtönungen, die außerdem noch in Abhängigkeit von der Konzentration der Aminosäuren Schwankungen unterworfen sind, Verwechslungen auftreten können, liegt auf der Hand. Außerdem muß noch bei dem eindimensionalen Chromatogramm mit kurzer Laufstrecke und schmalem Streifen, wie es von Anders angewandt wurde, mit Überlagerungen gerechnet werden, durch die die Farbtönungen ihren Wert verlieren.

Die Beweise, die Anders für die Gegenwart von Lysin, Histidin und Tryptophan im Reblausspeichel anführt, sind m.E. nicht stichhaltig, zumal auch der Nachweis mit Ninhydrin wegen der schwierigen Speichelbeschaffung nur an 2 eindimensionalen Chromatogrammen mit verkürzter Laufstrecke durchgeführt werden konnte, von denen eines in Phenol und das andere in Partridgegemisch (Butanol : Eisessig : Wasser = 4 : 1 : 5) aufsteigend chromatographiert wurde.

Bei der Suche nach Fehlerquellen in der eigenen Versuchsanordnung wurde noch daran gedacht, daß die mit dem Speichel in das Papier abgegebenen Aminosäuren in dem angefeuchteten Papier oxydativen Veränderungen unterworfen sein könnten und somit nicht der tatsächliche Aminosäuregehalt des Speichels erfaßt wird. Die Nachprüfungen mit Reinsubstanzen, insbesondere mit Tryptophan und Histidin, unter gleichen Versuchsbedingungen ergaben jedoch keine Anhaltspunkte dafür.

Eine Substanz, die regelmäßig mit Ninhydrin auf den Chromatogrammen nachgewiesen wurde, konnte nicht identifiziert werden. Zuerst wurde auf Grund des blauen Farbtones und des Rf-Wertes angenommen, daß es sich um β-Alanin handelt, doch konnte mit Reinsubstanzen keine Bestätigung dafür erbracht werden. Wahrscheinlich handelt es sich um ein Peptid. Eine einwandfreie Bestimmung ist jedoch erst dann möglich, wenn der Reblausspeichel hydrolysiert und anschließend chromatographiert wird. Diese Untersuchungen stehen jedoch noch aus.

DISKUSSION

Aminosäuren wurden in letzter Zeit mit Hilfe verfeinerter analytischer Methoden mehrfach im Speichel pflanzensaugender Insekten nachgewiesen. So fand KLOFT (1956) die Aminosäuren Glutaminsäure, Glycin, Alanin und Asparaginsäure im Speichel der siebröhrensaugenden Aphide *Myzus ascalonicus* Donc., sieben verschiedene Aminosäuren im Speichel der Wanze *Palomena prasina* L. und dreizehn Aminosäuren im Speichel der Wanze *Pyrrhocoris apterus* L. Bei der parenchymsaugenden Aphide *Liosomaphis abietina* Walk. wurden von KLOFT & EHRHARDT (1959) die Aminosäuren Alanin, Arginin, Asparaginsäure, Cystin, Leucin, Serin und Valin festgestellt und bei *Viteus vitifolii* Shim. — ebenfalls ein Parenchymsauger — von ANDERS (1957, 1958) die Aminosäuren Lysin, Histidin, Tryptophan und wahrscheinlich Glutaminsäure und Valin. In der vorliegenden Untersuchung wird der Befund von Anders bestätigt, daß der Reblausspeichel freie Aminosäuren enthält, doch wurden mit Ausnahme der Glutaminsäure andere ninhydrinpositive Substanzen identifiziert. Vor allem wurden Tryptophan und Histidin nicht gefunden, die nach Anders in Verbindung mit Glutaminsäure die cecidogenen Substanzen des Reblausspeichels ausmachen sollen. Zu dieser Auffassung gelangte Anders, weil die Kombination dieser Aminosäuren in Modellversuchen an jungen Rebwurzeln, aber auch an Zwiebelwurzeln, „Wurzelkeulen" erzeugen, die histologisch vollständig Reblausgallen gleichen.

Inwieweit diese Aminosäuren tatsächlich zur Wurzelkeulenbildung befähigt sind, kann auf Grund der eigenen Untersuchungen nicht beurteilt werden, doch muß nach den Ergebnissen der Speichelanalysen abgelehnt werden, daß Histidin und insbesondere Tryptophan die Hauptbestandteile der cecidogenen Substanz im Reblausspeichel darstellen. Damit soll jedoch nicht gesagt werden, daß Aminosäuren im Speichel der Insekten für die Gallbildung bedeutungslos sind. Im Gegenteil muß nach den Befunden von KLOFT (1956) über den Einfluß der Aminosäuren im Speichel siebröhrensaugender Insekten auf ihre Wirtspflanzen angenommen werden, daß gerade die Aminosäuren besonders physiologisch aktiv sind. Das geht auch aus den Befunden von STEINBERG (1949), STEINBERG, BOWLING & MC MURTREY (1950) und COMMONER & NEHARI (1953) hervor. Danach soll der Gehalt an freien Aminosäuren in der Pflanze deren Morphologie und Anatomie weitgehend beeinflussen.

Aus diesen Gründen muß auch in Zukunft den freien Aminosäuren im Speichel pflanzensaugender Insekten besondere Aufmerksamkeit gewidmet werden.

In diesem Zusammenhang ist noch die Frage nach der Herkunft der Aminosäuren im Speichel von Bedeutung. Durch zahlreiche Untersuchungen (AGRELL, 1949; AUCLAIR & PATTON, 1950; AUCLAIR & DUBREUIL, 1953; PRATT, 1950; CHEN & HADORN; 1954, usw.) ist bekannt geworden, daß der Aminosäuregehalt der Insektenlymphe relativ hoch ist — im Vergleich zum Säugetier z.B. 50—100 × höher (WIGGLESWORTH, 1955). Aus diesem Grunde ist nach KLOFT (1956) der Gedanke, daß Aminosäuren aus der Lymphe in den Speichel übertreten, nicht ungewöhnlich, zumal ja durch die Untersuchungen von WIGGLESWORTH (1943, zit. nach NUORTEVA, 1958), DAY & IRCZYKIEWICZ (1953), NUORTEVA & REINIUS (1953), NUORTEVA (1955) usw. bekannt geworden ist, daß radioaktive Nahrungsbestandteile, Enzyme (Papain), ja sogar Hämoglobinmoleküle und Viren aus dem Darm über die Lymphe in den Speichel gelangen können.

Bei der Reblaus könnten die Aminosäuren und Amide den gleichen Weg gehen. Im Körper der Reblaus treten sie jedoch in anderen quantitativen Verhältnissen auf als im Speichel. Ganz besonders gilt das für die Amide Asparagin und Glutamin. Während beide Verbindungen bei jungen, frisch aus den Eiern geschlüpften Rebläusen im Vergleich zu den anderen ninhydrinpositiven Substanzen nicht besonders in Erscheinung treten, steigt die Konzentration bei erwachsenen Rebläusen mindestens auf den doppelten Wert, und im Speichel der erwachsenen Rebläuse überwiegen beide Amide in auffallender Weise.

Amide treten sowohl in der Pflanze als auch im Tier als physiologisch weniger aktive Stickstoffverbindungen auf. In der Pflanze dienen sie häufig als Stickstoffreserve in Speicherorganen, bei bestimmten Aphiden möglicherweise bevorzugt der Stickstoffausscheidung. MALTAIS & AUCLAIR (1952) untersuchten den Honigtau der Aphide *Myzus circumflexus* (Buck.) und fanden neben 20 verschiedenen Aminosäuren vor allem die Amide Asparagin und Glutamin. Asparagin tritt in der weitaus höchsten Konzentration auf. Wird nun, wie bei der Reblaus, im Verlauf der Entwicklung zum erwachsenen Tier die Afteröffnung geschlossen, so werden offenbar die Exkrete in Verbindung mit anderen Körperfunktionen abgegeben, z.B. bei der Eiablage (GERSCH, 1953; HALLE, 1959) oder mit dem Speichel.

Damit wird die Auffassung von Anders unterstützt, daß die freien Aminosäuren, die von den Aphiden in das Wirtsgewebe abgegeben werden, Exkretionsprodukte des tierischen Organismus darstellen. Sie gelangen offenbar über die Lymphe in die Speicheldrüsen und werden mit dem Speichel ausgeschieden.

Hinsichtlich des Ausscheidungsweges vermutet allerdings Anders, daß diese Verbindungen nicht unbedingt im Sekret der Speicheldrüsen enthalten sein müssen, obwohl er in seinen Arbeiten von Speichelanalysen spricht und außerdem annimmt, daß das Speichelrohr die Exkrete nach außen leitet. Wie das unter Umgehung der Speicheldrüsen möglich sein soll, wird nicht gesagt. Möglicherweise nimmt Anders tatsächlich im Zusammenhang mit der Nahrungsaufnahme der Aphiden eine regelmäßige Regurgitation an. Die Spaltprodukte der Nahrung müßten dann direkt aus dem Darm in die Pflanze zurückbefördert werden, was allerdings nicht über das Speichelrohr (= hintere Rinne der Maxillarborsten) wie Anders schreibt, sondern nur über das Nahrungsrohr (= vordere Rinne der Maxillarborsten) erfolgen könnte.

Nach KLOFT (1956) hat der Speichel bei parenchymsaugenden Insekten gleichfalls Exkretionsfunktion. Im Gegensatz zu Phloemsaugern muß der Speichel dieser

Aphiden die Voraussetzung für eine kontinuierliche Diffussion von Nahrungsstoffen zur Rüsselspitze hin schaffen. Es ist deshalb nach Kloft eine Dauersekretion erforderlich, die gleichzeitig einen Teil der Wasserexkretion übernimmt. Mit der größeren Flüssigkeitsmenge gelangen offenbar auch größere Mengen physiologisch wirksamer Verbindungen in die Pflanze, die ihrerseits die im allgemeinen größere Schädlichkeit parenchymsaugender Insekten zur Folge haben können.

In diesem Zusammenhang muß aber sicherlich noch als zweiter Faktor berücksichtigt werden, daß sich die Inhaltsstoffe des Speichels siebröhrensaugender Aphiden schneller in der Pflanze verteilen als bei Parenchymsaugern. Durch den langsameren Abtransport der Speichelinhaltsstoffe und durch die größeren Speichelmengen der Parenchymsauger könnte es dann im Stichbereich relativ schnell zur Anhäufung physiologisch wirksamer Substanzen kommen und in deren Folge zu lokalen Schädigungen, z.B. Gallen oder Nekrosen.

Herrn Dr. W. Kloft möchte ich auch an dieser Stelle für die freundliche Überlassung eines Exemplares seiner Habilitationsschrift danken.

SUMMARY

THE AMINO ACID CONTENT OF THE SALIVARY GLAND SECRETION OF THE VINE PHYLLOXERA, VITEUS VITIFOLII *SHIMMER, HOMOPTERA*

Saliva was collected from the adult phylloxera, by inducing them to pierce lightly-damped chromatographic paper with their probosces. The saliva is deposited on the paper around the puncture. Adult phylloxera are especially suitable for this method of obtaining saliva, because closure of the anus during the course of development leads to the interruption of excretion by that route in the adult. Then the saliva was analysed by means of paper chromatography. On each two-dimensional chromatogram the saliva of about 700 phylloxera was collected, and its amino acid, amide and peptide content determined. The amino acids alanine, glutamic acid, glycine, serine and arginine, and the amides asparagine and glutamine, were clearly identified. Asparagine and glutamine appeared in much higher concentrations than the amino acids. One other substance was found, and this may possibly prove to be a peptide. Anders' report, that phylloxera saliva contains the amino acids tryptophane, histidine, lysine and possibly glutamic acid and valine, is not substantiated.

The amino acids, and particularly the amides, are considered to be excretory products of the adult phylloxera, which reach the salivary glands by way of the lymph, and are eliminated in the saliva.

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FURTHER STUDIES ON THE REPRODUCTIVE ISOLATION OF GEOGRAPHICAL STRAINS IN THE *TETRANYCHUS TELARIUS* COMPLEX

BY

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Cross breeding experiments with British and North American colour forms of the mites included in the *Tetranychus telarius* complex suggest that *T. urticae* Koch is the correct name for the green form, and *T. cinnabarinus* Boisd. for the reddish form. Different strains, with different degrees of incompatibility, occur in each species.

Recently, it has been suggested that the 'two spotted spider mites' which are included in the *Tetranychus telarius* complex include several distinct species and subspecies which, though morphologically indistinguishable, will not hybridize. They can be divided into two main groups: those possessing carmine or reddish-brown summer females and those with green summer females. The males of both groups are indistinguishable by colour, varying from straw yellow to pale green.

In cross-breeding tests, BOUDREAUX (1956) found that hybrid females were readily obtained by reciprocal crosses between the carmine and green forms of North America but that such hybrids were sterile or produced only a small number of male progeny. He considered them to be distinct species, referring the carmine form to *Tetranychus cinnabarinus* Boisd. and retaining *T. telarius* L. for the green.

HUSSEY & PARR (1958) obtained similar results in cross-breeding tests between the reddish-brown and green forms occurring in British glasshouses. For the time being, they are both included under the specific name *urticae* Koch, to which the European forms of this mite have recently been delegated[1]).

DILLON (1958) failed to obtain hybridization between several red and green forms from different geographical locations in Europe and N. America. He concluded that the German and British forms were distinct from one another as well as from those of N. America, and that there were also some distinct American red species. He divided the American green form into two distinct geographical sub-species.

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[1]) At the time of the publication of our 1958 paper we were unaware of the change of opinion by BOUDREAUX (1958) regarding the onset of diapause in American *T. cinnabarinus*. As VAN DE BUND & HELLE (1960) point out in the following paper there is now no valid biological reason for regarding the American and European forms as distinct. In the present paper we have concurred with this view despite the fact that the particular strains tested by us were not interfertile.

Our experience with *T. cinnabarinus* in England agrees with the observations recorded by van de Bund & Helle but it is perhaps worthy of record that, at our Institute, the species seems to have established itself naturally on tomatoes and cucumbers as well as on the perennial type of glasshouse crops such as carnations and arum lilies with which it is most usually associated (Note added to the proof).

The present paper deals with cross-breeding tests between the British and some strains of the N. American colour forms in a further attempt to clarify their true relationship.

MATERIAL

The American material comprised colonies of the Beltsville red form reared originally from cucumber under glass and identified as *T. cinnabarinus* Boisd. by BOUDREAUX (TAYLOR & SMITH 1956), and the 'Massachusetts' and 'Cranbury' strains of the green *T. telarius* L. bred originally under glass on carnations and roses respectively. These green strains were shown by TAYLOR & SMITH (1956) to interbreed freely though they exhibited marked differences in their susceptibility to organic-phosphorus acaricides.

The British colour forms used in the tests had been bred in isolation through many generations on French bean. They are normally inhabitants of glasshouses, breeding on tomato, cucumber, carnations and many other ornamental plants.

METHOD

As virgin females produce only male offspring, the mite stocks for testing were started by mating such a female with one of her sons and continued by inbreeding. The stocks were maintained on French bean plants isolated in cages and standing in a large trough of water which decreased the risk of contamination to a minimum. The mites used for testing were transferred to leaf discs cut from similar uninfested plants and floated upon nutrient solution in petri dishes. To ensure that no prior mating had taken place, one female in the deutonymph resting stage and one mature male were transferred to each disc.

The experiments were done in an incubator at 24° C and a daily photoperiod of 16 hr. was maintained throughout.

RESULTS

A total of 39 crosses, including reciprocals, was made between the three green forms and 22 between the two reddish forms. No difficulty was experienced in obtaining first generation eggs in any of the crosses.

Crosses between the British green *urticae* and the Massachusetts and Cranbury green strains of *telarius,* produced F1 hybrid females, which though fully fertile, showed a certain degree of incompatibility (Table I). For example, crosses between *urticae* females and American males produced only 5 eggs/♀/day in the first generation, though a high proportion of them were viable. Under the conditions of temperature prevailing, an average of at least 10 eggs/♀/day is normally to be expected within strains of this species (HUSSEY & PARR 1957). Furthermore, there was a preponderance of male progeny.

These species are arrhenotokous, so that virgin females produce only males, while mated fertilised females, produce both male and female offspring. The production of a greater proportion of males therefore, indicates a possible lack of compatibility between the male sperm and female ova.

TABLE I

Results of cross-breeding tests between British and N. American colour forms in the T. telarius *complex*

Cross	Generation	No. Pairs	Eggs Laid					Adults Matured (Sample only)		Sex Ratio
			Total	Mean	Range	♀/Day	% Mean viability	♀	♂	♀ : ♂
Green forms										
British ♀ × Massachusetts ♂	Original	9	249	28	14— 55	3.6	—*	42	57	1 : 1.4
	F_1	10	808	81	27—130	9.2	47	95	129	1 : 1.4
Massachusetts ♀ × British ♂	Original	9	388	43	14— 73	7.2	—*	88	40	2 : 1
	F_1	9	817	91	37—135	11.8	39	164	50	3 : 1
British ♀ × Cranbury ♂	Original	11	298	27	12— 52	4.6	—*	55	83	1 : 1.5
	F_1	9	585	65	22—118	11.8	39	27	104	1 : 4
Cranbury ♀ × British ♂	Original	10	312	31	10— 61	8.0	—*	91	51	2 : 1
	F_1	13	726	55	11—130	9.2	49	205	60	3 : 1
Red forms										
Beltsville ♀ × British ♂	Original	9	952	106	22—210	9.5	—*	263	89	3 : 1
	F_1	20	295	15	0— 63	1.6	4	0	1	—
British ♀ × Beltsville ♂	Original	13	1321	102	37—218	12.5	—*	266	109	2.5 : 1
	F_1	18	59	3	0— 14	0.3	0	0	0	—

* Accurate figures were not obtained but it is known they exceeded 70%

In the F1 generation, the fecundity and rate of oviposition of the hybrid females were considerably higher than in the initial cross though fewer than half the eggs were viable. Again the number of male progeny exceeded that of the female.

The reciprocal crosses showed little loss of fecundity though the F1 hybrids again produced a high proportion of non-viable eggs. The proportion of female to male progeny in both the original and F1 generations was about 2 : 1, a ratio considered normal for this group of mites.

The crosses between the British reddish-brown *urticae* and the Beltsville red *cinnabarinus* produced F1 hybrid females which were either sterile or of very low fertility. The few eggs obtained from these hybrids were mostly small and dark brown in colour. Of the few larvae which hatched from them, only one, a male, succeeded in completing its development.

DISCUSSION

The green forms hybridized freely, but inconsistences in the sex ratio suggested that the sperm of the American males could not move successfully from the spermatheca to fertilise the eggs developing in the ovary of the British females. On the other hand, sperm from British males successfully fertilised developing eggs in American females. Thus the crosses between British and American green forms suggest that they are the same species, but that different degrees of incompatibility exist between them. BOUDREAUX (in litt.) conducted many cross-breeding tests on strains from America, Britain, Germany and Japan, and found that all showed different affinities with each other: indeed, two strains which appeared to be incompatible could both be compatible with a third strain.

In the past, much difficulty has arisen over the synonymy of this mite in Europe. At the Symposia on Acarology held at Wageningen in 1956 and at East Malling in 1959, it was agreed that the specific name *urticae* Koch be used in preference to *telarius* L. The experimental studies referred to in this paper suggest that *urticae* is the correct name for all green forms in the *telarius* complex. *T. bimaculatus* Harvey therefore becomes a synonym of *T. urticae* Koch.

In the red forms, however, although the behaviour of the sperm was normal, the genetic make-up was sufficiently distinct as to suggest specific differences. The almost complete intersterility existing between the two forms strongly suggests that they are distinct species. Despite such incompatibility they produced a perfectly normal first generation, both in regard to fecundity and sex ratio of the progeny.

BOUDREAUX (in litt.) however, has shown that the British form is very similar to that occurring in Japan which itself is compatible with that from Germany which DILLON (1958) had claimed to be specifically different from the British. Dillon based his conclusions on biological differences, whereas Boudreaux's experiments showed them to hybridize normally.

The inference is that all these red forms are local strains of the same species, *T. cinnabarinus* Boisd.

Possibly both colour forms originated from a single source and became isolated geographically by commerce. In modern times hybridization between these isolated strains has taken place, and different degrees of relationships have become extant, which cannot be correlated with geographic or host differences. For instance, Boudreaux (in litt.) claims that he has two stocks of green forms from the city of Baton Rouge, Louisiana, taken on different host plants, which are less compatible with each other than either is with Japanese green stocks.

No consistent morphological differences have been established and the cross-breeding experiments referred to above emphasise that all strains within each colour group, should be regarded as the same species. The two species *T. urticae* and *T. cinnabarinus* are evidently polytypic in the sense used by Pritchard & Baker (1955) in their definition of *T. telarius.*

The complex relationship between the strains of *T. urticae* demonstrated in recent years, emphasizes the importance of using pure strains of known genetic constitution, in any experiments with these mites.

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ZUSAMMENFASSUNG

WEITERE UNTERSUCHUNGEN ÜBER DIE GESCHLECHTLICHE ISOLATION GEOGRAPHISCHER POPULATIONEN IM TETRANYCHUS TELARIUS-*KOMPLEX*

Es würden Kreuzungsversuche durchgeführt, um die Verwandtschaft zwischen britischen und nordamerikanischen Farbformen des sogenannten *T. telarius* Komplex zu prüfen.

Paarungen zwischen Vertretern der grünen Form ergaben F_1 Hybriden, die voll fortpflanzungsfähig waren, obgleich sie unterschiedliche Grade von Unverträglichkeit zeigten. Solche zwischen den rötlichen Formen ergaben vollständige Unverträglichkeit; die F_1 Weibchen waren steril oder hatten sehr geringe Fruchtbarkeit. Wenn Nachkommen von derartigen Weibchen erzeugt wurden, waren sie männlichen Geschlechts.

Die unterschiedlichen Verwandtschaftsverhältnisse, die zwischen Farbformen verschiedener geographischer Verbreitung bestehen, werden besprochen, und es wird daraus geschlossen, dass alle Angehörigen einer jeden Farbgruppe als gleiche Arten betrachtet werden müssen. Es wird vorgeschlagen, dass *T. urticae* Koch der korrekte Name ist, der jetzt für die grünen Formen angewendet werden sollte, und *T. cinnabarinus* Boisd. für die rötlichen Formen beizubehalten.

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INVESTIGATIONS ON THE *TETRANYCHUS URTICAE* COMPLEX IN NORTH WEST EUROPE (ACARI: TETRANYCHIDAE)

BY

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Two species of spider mite occur in greenhouses in the Netherlands.

Tetranychus urticae Koch is usually green, can live on many plants, but cannot build up large populations on carnations; it may have a diapausing stage which is very resistant to cold. No biological races of *T. urticae* were found.

T. cinnabarinus Boisd. is carmine-coloured, often found on carnations, and does not have a diapause. It is not resistant to cold.

The two species do not interbreed. Growers of carnations have only *T. cinnabarinus* to deal with, and can arrange control measures accordingly.

For a long time the validity of descriptions of new species of Tetranychidae was based on morphological characteristics only. Inadequacy of this work was illustrated best by the study of PRITCHARD & BAKER (1955) who give no less than 58 synonyms for the species *Tetranychus telarius* (L.) (= *T. urticae* Koch).

Later, biological characteristics were also used to distinguish between species and some species were redescribed. Important results were obtained by mating experiments, as the validity of two species can be established by the lack of interbreeding. BOUDREAUX (1956) gave a revision of *Tetranychus telarius* and, following these modern methods, distinguished three species among the strains of this complex, existing in the U.S.A.: *T. telarius, T. cinnabarinus* Boisd. and *T. lobosus* Boudreaux. These descriptions are based also on mating experiments. DILLON (1958) investigated sixteen geographic strains of the *T. telarius* complex originating from Europe as well as from North America and his mating experiments proved lack of conspecifity between the european strain which is called *T. telarius* and the American strain, which he named *T. bimaculatus* Harvey. The species *T. cinnabarinus,* which is mainly characterized by the carmine colour of the summer females, was found also to occur in Europe.

HUSSEY & PARR (1958) made a genetic study of the colour forms of *T. urticae* in greenhouses in England. Two distinct forms are known, mainly differing by the colour of the summer females, a green one and a brownish-red one. Different behaviour in winter time is mentioned, as the green form enters diapause, which is governed by photoperiod, temperature and food (LEES 1953) while the brownish-red form has no diapause and dies in England during winter time in out-door conditions. Hussey & Parr are of the opinion that this red form cannot be identical with *T. cinnabarinus*, as the biological data mentioned by Boudreaux are different from those for the red form in England. According to them, DILLON (1958) is right in considering that the forms from North America and England are not identical, though evidence can be given only by mating experiments.

In the greenhouses in North West Europe also, several forms of spider mites of the *T. urticae* complex have been known for some time. It seemed promising to investigate these forms once more. Special attention was paid to the form which occurs on carnation (*Dianthus caryophyllus* L.). The urgency for more fundamental work is increased, since recent results suggest that chemical control measures may fail in the near future due to the development of resistant forms (HELLE, 1959).

MORPHOLOGY AND TAXONOMY

Many species of spider mites found on different host plants in the Netherlands have been described by OUDEMANS (1927-'31) and a review was given by GEYSKES (1939). Other species were described from different parts of Europe. However, the recent investigations of McGREGOR (1950) and PRITCHARD & BAKER (1955) showed that most of the descriptions of Oudemans are overruled by new findings. The characteristics on which the descriptions had been based, are nowadays considered to have less taxonomic value. The optical equipment used was less perfect and therefore several important characters escaped notice of the acarologist, for instance the importance of the morphology of the aedeagus was misjudged.

It appeared that in the greenhouses two distinct forms could be easily distinguished, one of them having green-coloured summer females, while those of the other forms are carmine-coloured (Fig. 1). A complete description of the morphology of these two forms will not be given here, as this has been done before (GASSER, 1951). Only the principal characters in which both forms show differences will be mentioned.

The observations were made with a Dialux microscope E. Leitz-Wetzlar; ocular periplan 10 x; objective 170/o.17, Pv apo öl 90 A 1,10 (1,25 x), under phase contrast and oil immersion.

Description of the green form. Ground-colour of living females pale yellowish-green or amber, varying to dark green, dependent on the food-condition (colour 5.0 IJ 7/6 - 7.5 IJG 6/8, MUNSELL, 1952). Dorsolaterally on each side, a large black spot is found, often divided into two or three smaller ones; size depends also on condition of food. In summer, starving females have an amber tint, frequently accompanied by an orange-like glow. In that case, side-spots are reduced and often subdivided, mostly brownish. Females in diapause bright pale orange (5 IJR 7/10), dark side-spots lacking.

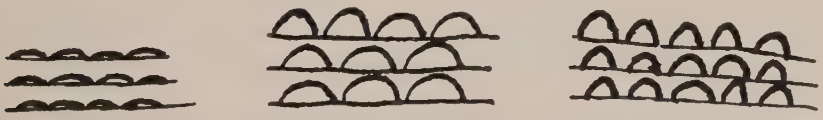

Fig. 2. Variability of the structure of the dorsal integumentary striae of the active feeding summer female of *T. urticae*. Left: less developed; middle: normal; right: strongly developed.

Lobes of dorsal integumentary striae of feeding females very variable in structure (Fig. 2). In case of strong development, individual lobes semi-oblong to almost semi-circular; in case of slight development, lobes more semi-oblong with more flattened upperside. Development of these structures very slight in many specimens, in that case individual lobes hardly recognizable. Females in diapause have perfectly smooth integumentary striae.

Tarsi of first pair of legs with about 16 - 17 setae (Fig. 3). 4 - 5 setae proximad of proximal duplex setae. Tibiae of the first pair of legs with 10 setae. Females with extra setae on first tarsi not found in the Netherlands.

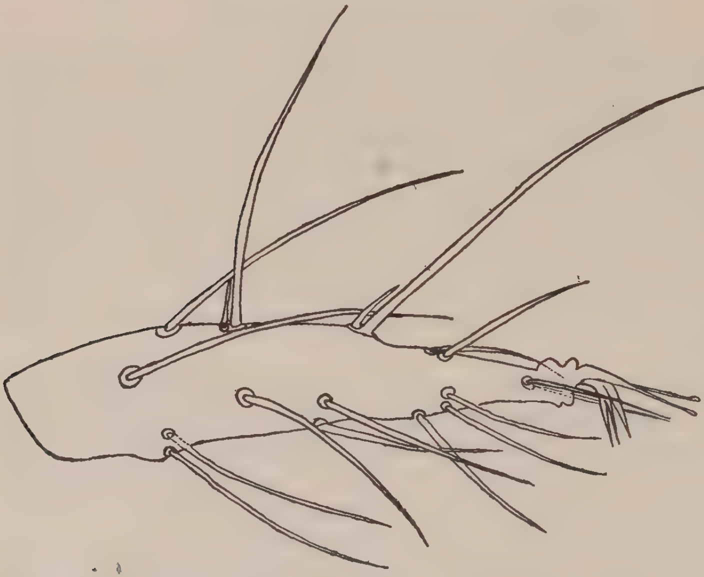

Fig. 3. Tarsus I of female of *T. urticae.*

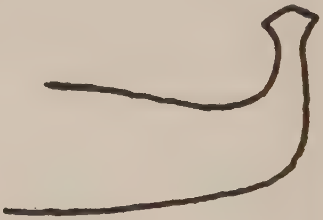

Fig. 4. Aedeagus of *T. urticae.*

Living males pale yellowish green to light amber, with sometimes a slight orange-like tint. Knob of aedeagus dorsally strongly convex, inner projection acute, forming generally an angle with the hook of more than 140°; outer-lobe also tapering acutely (Fig. 4).

Eggs translucent and shining. Newly-born larvae pale-amber; as soon as feeding starts, becoming pale yellowish-green to green and with dorsolateral spots appearing. Nymphs have same colour as adults. Newly-moulted specimens as a rule amber-coloured with slight indication of dark spots, which are mostly coloured pale-brown.

Our preliminary conclusion is that this form can be named *Tetranychus urticae* Koch (= *T. telarius* (L.)).

Description of the carmine form. Newly metamorphosed females pale-amber coloured with orange tint, dorsolateral side-spots pale-brown. Colour darkens with advancing age. Females in full egg-production gradually dark brownish-red (10 R - IJR 5/10 - 3/4). At that stage side-spots very large and black. Non feeding females or specimens exposed to low temperatures, brightly orange (5 IJR 7/10). As time elapses since feeding, side-spots become smaller. Lobes of dorsal integumentary striae of active feeding females as a rule well developed (Fig. 5). Individual lobes of this structure mostly semi-circular to somewhat semi-oblong. In case of strong development lobes often triangular in form. If less developed, lobes more oblong, and in that case not distinguishable from those of green form. Hibernating females with perfectly smooth integumentary lobes, showing no differences from the green form either in colour, or in morphological characters.

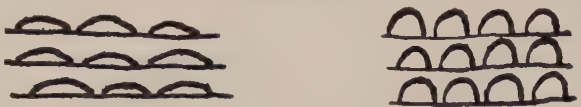

Fig. 5. Variability of structure of the dorsal integumentary striae of the female of *T. cinnabarinus*. Right: normal; left: less developed.

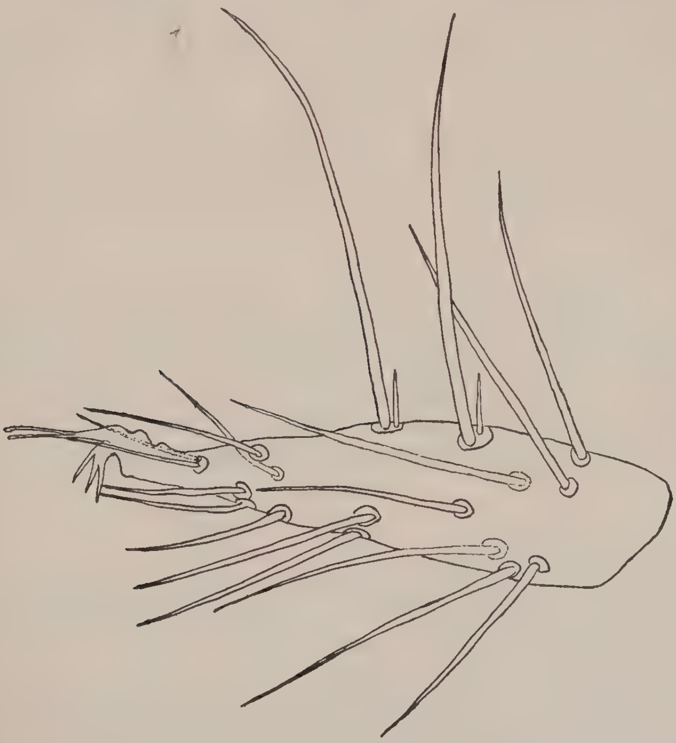

Fig. 6. Tarsus I of female of *T. cinnabarinus* with the extra setae proximad of the proximal duplex setae.

Tarsi of the first pair of legs of most females with 16 - 17 setae[1]) (Fig. 6). Specimens with 18 - 19 setae often found. 4 - 5, frequently 6 - 7 setae proximad of the proximal duplex setae of the tarsi of the first pair of legs. Tibiae of first pair of legs with about 10 setae, in case of extra setae, 13.

Living males amber to bright orange-like red with fine dark pigmental spots dispersed all over the body. Knob of aedeagus dorsally convex, with some specimens strongly convex; inner-projection obtuse to rounded, forming mostly an angle of about 90° with the hook, often larger than the apically acute outer-projection. Shaft sometimes dorsally strongly convex (Fig. 7).

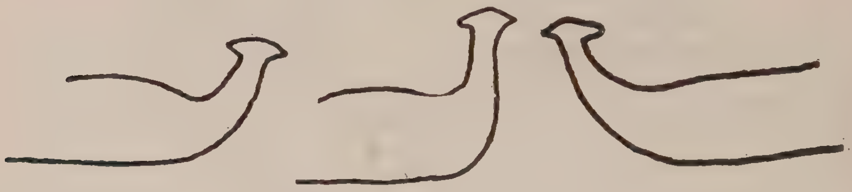

Fig. 7. Different forms of aedeagus of *T. cinnabarinus*.

Eggs translucent with a pink to reddish tint, darkening with proceeding development. Newly-born larvae pale-amber, if feeding on carnation soon becoming orange; then dark side-spots appear. If feeding on beans, ground-colour becomes light green with dark side-spots. On carnation, nymphs orange-like green coloured with brownish to black side-spots; on bean green coloured, carmine colouring appearing not before the adult stage. On bean teleiochrysali with pale brownish-orange tint, contrary to those of the green form.

Though comparison with material of North America was not yet possible, we think to have enough arguments both from morphological data, and from biological facts, to be described below, to name this carmine form *Tetranychus cinnabarinus* Boisd., 1867.

FREE MIGRATION AND COLONISATION BETWEEN DIFFERENT HOST PLANTS

Many research-workers, among others LINKE (1953) have proved that mites of the *T. urticae* complex are very polyphagous. From those statements it is not evident, however, that mite populations from different host plants have been tested reciprocally in all combinations. It might be supposed that on certain plants biological races have been formed that could no longer develop on other host plants.

Materials and Methods

Mite populations from different plants were taken. Among them were three populations from cultivated plants viz: glasshouse-rose (cv. Roselandia) from Aalsmeer, French bean (*Phaseolus vulgaris* L.) from the field at Wageningen, and carnation (*Dianthus caryophyllus* L.) from Aalsmeer. As to the wild plants, we used mite populations from *Chelidonium majus* L. (Wageningen, Lisse), *Euonymus europaeus* L. (Wageningen) and *Rosa canina* L. (Wageningen). It is known, that these plants are very often infested by spider mites. We also used mites from *Sambucus nigra* L. (Aalsmeer), *Althaea rosea* Cav. (Wageningen) and *Urtica dioica* L. (Aalsmeer), the mites of which plants have been described as separate species by former authors.

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[1]) Every duplex seta is counted as one.

The mites from carnations belonged to the species *T. cinnabarinus,* the forms from the other host plants were all *T. urticae*.

Adult females from nine different host plants were transferred to leaves of eight different host plants, so that seventy-two combinations of mite populations and host plants were obtained.

A first requirement of this experiment was a reliable isolation of the different populations. Therefore ten adult females were placed on a leaf-disc, cut from a certain host plant. The leaf discs were placed on water-soaked cotton-wool, situated in the middle of a petri-dish. The experiment was carried out in tenfold, each dish containing five replications. When needed the offspring was supplied with fresh leaf discs. The whole experiment was placed in a glass show-case, under 24 hours light by fluorescence-lamps, to suppress diapause. During the experiment the temperature was kept at about 26 °C.

Results

Eggs were deposited in all cases, and a second generation was bred. By continuous breeding two groups could be distinguished, differing by the fact if the offspring of the second generation could reproduce further or not. The groups are indicated by + and — in Table I. It appears, that spider mites from rose, bean, *Chelidonium, Urtica, Sambucus* and *Althaea* developed normally on all host plants included in the study, with the exception of carnations. All these populations contained green forms (*T. urticae*) only. The carmine coloured mites (*T. cinnabarinus)* appeared to develop normally on all host plants used including carnation.

TABLE I

Possibilities of forming colonies on several hostplants.

Hostplants: Mite populations from:	Carnation	Rose	Bean	Sambucus	Urtica	Althaea	Chelidonium	Euonymus
Carnation	+	+	+	+	+	+	+	+
Rose (glasshouse)	—	+	+	+	+	+	+	+
Rosa canina	—	+	+	+	+	+	+	+
Bean	—	+	+	+	+	+	+	+
Sambucus	—	+	+	+	+	+	+	+
Urtica	—	+	+	+	+	+	+	+
Althaea	—	+	+	+	+	+	+	+
Chelidonium	—	+	+	+	+	+	+	+
Euonymus	—	+	+	+	+	+	+	+

+ = Normal development and propagation of the offspring
— = Mortality during the development of the offspring

INFLUENCE OF THE FEEDING ON CARNATION AND BEAN ON FERTILITY, POSTEMBRYONIC DEVELOPMENT AND MORTALITY

In numerous cases, in which we observed spider mite infestations in different carnation-houses, either in the Netherlands or in Belgium (Aalst) or Germany (Frankfurt, Kleve, Xanthen), they always appeared to be caused by *T. cinnabarinus.* Hussey & Parr (1958) too mentioned that in England the carmine-coloured form of *T. telarius* is usually associated with carnations.

This question was studied more closely in the following experiments where the development of *T. urticae* and *T. cinnabarinus* was compared on two host plants, carnation and bean (*Phaseolus vulgaris* L.).

Egg Production

Separate cultures of both species on carnation and on bean were started from which adult impregnated females were taken for determination of egg production. In the case of *T. urticae* on carnation, however, this was impossible, as the larvae could not be reared on this host plant in sufficient quantities. For this reason *T. urticae* was cultivated on carnation only from the deutonymph-stage on.

The adult mites were isolated in separate small plastic cages (Meltzer, 1955) (Fig. 8). Every other day the mites were transferred to another cage and the eggs were counted. The experiment was carried out in a space in which the temperature was kept at 26° C, and 24 hours of light were given. The results are given in Table II.

TABLE II

Egg production of T. urticae *and* T. cinnabarinus *on bean and carnation*

	Number of females	Host plant	Average per ♀	Maximum per ♀	S.D.
T. urticae	12	bean	123.3	178	31.3
,,	12	carnation	15.8	42	10.4
T. cinnabarinus	12	bean	107.4	158	25.8
,,	12	carnation	89.5	128	20.6

We may conclude from the difference in egg-production of *T. urticae* on bean and carnation respectively that carnation, also to the imago, is unsuitable as a host plant ($\frac{\bar{v}}{\sigma_{\bar{v}}} = 18$). No differences were found in egg production of *T. cinnabarinus* on both plants. The slightly higher egg production of *T. urticae* on beans in comparison with *T. cinnabarinus* is not significant.

Post-embryonic development

Populations of *Tetranychus cinnabarinus* show a considerable variability in morphological features, especially in the form of the aedeagus (Fig. 7). Sometimes it was found that a certain type of aedeagus occurred in one population, inhabitating a distinct glasshouse, and that another type, originating from another source, differed from the first mentioned. Two such populations which could be considered as extremes in morphological characteristics were chosen, and bred separately. They were named *Cinnabarinus* 1 and *Cinnabarinus* 2.

The development of these two populations was determined on carnation and on

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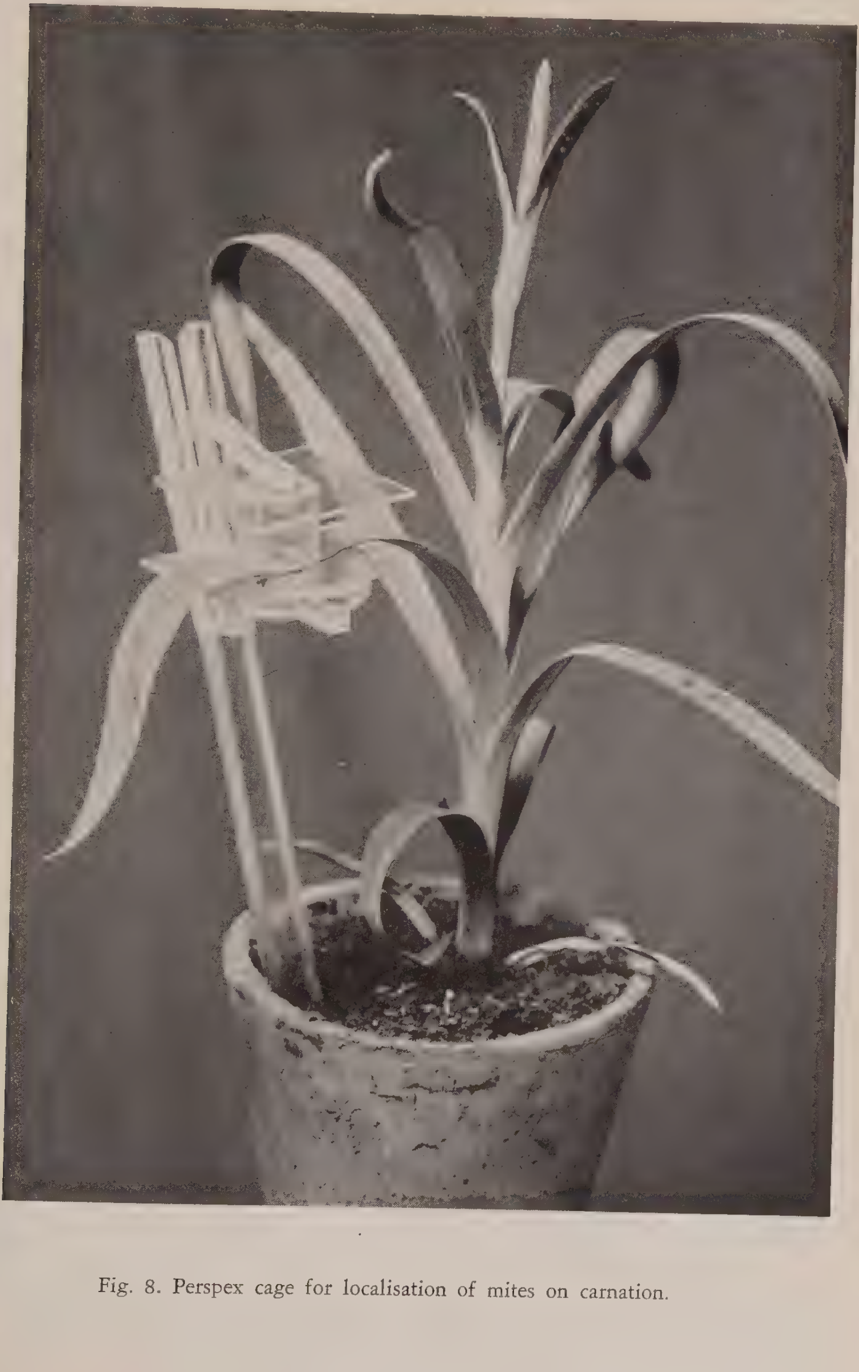

Fig. 8. Perspex cage for localisation of mites on carnation.

beans. Plastic cages were attached to the leaves of the two test-plants (Fig. 8) on places where a number of eggs were present. The cages were inspected at 12 hour intervals (daily at 9.00 and 21.00), at three different temperature conditions which were kept constant within 0.5 ° C.

TABLE III

Duration in days of the postembryonic development of T. cinnabarinus

	carnation			bean		
	20° C	25° C	30° C	20° C	25° C	30° C
T. cinnabarinus 1	11	7	4½	8	5½	3½—4
T. cinnabarinus 2	11	7	4½	8	5½	3½—4

Each figure is related to 8—22 individual tests

Although variations of development between individual mites occurred, these were small and fell within the 12 hour period between the observations (Table III).

Development on bean is considerably quicker than on carnation, at each of the three chosen temperatures.

Mortality

In order to determine the mortality of *T. urticae* and *T. cinnabarinus,* young bean- and carnation plants were used, on which a known number of eggs had been deposited. The plants were carefully isolated by placing them separately in water. Each group *(T. urticae* on carnation and bean, *T. cinnabarinus* on carnation and bean) was placed separately in a show-case. During the experiments the temperature was 25° C ± 1° C, while the relative humidity varied from 35 to 60 %. The plants grew in 24 hours light (5000 lux). Mortality was calculated from the difference between the number of eggs and the number of adults obtained. Immediately after the daily observations the adults were removed. The results of the experiment are shown in Table IV.

TABLE IV

Mortality of T. urticae *and* T. cinnabarinus *on bean carnation*

	Host	Number of eggs	Number of adults developed	Mortality %
T. urticae	Bean	831	815	2
T. urticae	Carnation	167	11	93
T. cinnabarinus	Bean	525	500	5
T. cinnabarinus	Carnation	537	506	6

The mortality of *T. cinnabarinus* is obviously low, on both bean and carnation. With *T. urticae* mortality is very high on carnation, as more than 90% of the larvae died without having taken food. This could easily be confirmed by the lack of side-spots in the larvae, which only appear after the first feeding.

T. urticae cannot multiply on carnation, chiefly because of decreased egg-productivity and high mortality among the newly hatched larvae. For *T. cinnabari-*

nus bean seems to be the more favourable host plant, as appears especially from the relatively slow development of the juvenile stages on carnation.

HIBERNATION AND COLD RESISTANCE OF T. URTICAE AND T. CINNABARINUS

From observations in the field it appeared that there is a real difference between the two species in mode of hibernation.

In *T. urticae* an obvious change in colour takes place in autumn. The adult females alter from green to pale-orange, while the side-spots disappear entirely. This orange winterform of *T. urticae*, even at higher temperatures, does not show any sucking-activity, and so does not take any food and does not produce eggs. At continuous low temperatures the majority of these winterforms seeks shelter to hibernate. At that time they are to be found in small clusters on dry stalks, in the crevices of the bark of trees, under withered leaves or in the soil. These spider mite colonies, closely herded together, often cover their shelter place with a close webbing. Apart from the fact of the colour change, the disappearance of the lobes of the dorsal striae of the cuticula in the winterform is remarkable.

In *T. cinnabarinus* we observed an entirely different habit of hibernation. Though here also a colour-change took place in autumn from carmine to orange, there proved to be no question of a real diapause. It appears that the orange-coloured spider mites of *T. cinnabarinus* start their activities again within a few days when brought under favourable conditions. Their original carmine colour comes back, and feeding and egg-production become more or less normal.

Moreover, there is a difference in behaviour. Unlike the diapause-form of *T. urticae,* the winterform of *T. cinnabarinus* does not show a tendency to find shelter, but remains on the host plant, i.e. the leaves. Even in the middle of the winter we found them in large numbers, unsheltered and close together, near the veins of partly decayed leaves.

The difference in hibernation habit which we observed between *T. cinnabarinus* and *T. urticae* led to experiments to try to induce diapause in both species under laboratory-conditions. Both species were cultivated from the larval stage in five thermostats, respectively at temperatures of 22.5, 20, 17.5, 15 and 12.5° C. The mites were given a daily photoperiod of 7 hours by means of fluorescence-lamps (2200 lux). They were cultivated on bean on leaf discs. In order to ascertain whether the mites had entered diapause, the adult females were placed on bean plants at a temperature of 25° C after different periods.

TABLE V

The influence of different temperatures on the percentages of females entering diapause, at a photoperiod of 7 hours. Average number of mites per experiment 32.

Temperature °C	22.5 ± 0.5		20.0 ± 0.5		17.5 ± 0.5		15.0 ± 0.5		12.5 ± 0.5	
Generation	P_1	F_1	P_1	F_1	P_1	F_1	P_1	F_1	P_1	F_1
T. urticae	0	0	61	50	95	—	100	—	100	—
T. cinnabarinus 1	0	0	0	0	0	0	0	0	0	0
T. cinnabarinus 2	0	0	0	0	0	0	0	0	0	0

In case the mites began feeding and egg-laying, and when the offspring also reproduced, it was clear that no diapause was induced (Table V, 0). If the

mites did not resume their activities within five days, diapause was indicated.
The difference between the species is quite evident; at lower temperatures *T. urticae* enters into diapause, while diapause fails to turn up in the two populations of *T. cinnabarinus*.

In another experiment the resistance to cold of the diapause-form of *T. urticae* and the hibernating form of *T. cinnabarinus* was compared. Both species were kept at 3°, -5°, -16°, and -22° continuously, observed daily and the number of survivors was counted. Table VI shows that *T. urticae* is more resistant than *T. cinnabarinus*.

TABLE VI

Resistance to cold of hibernating forms of T. urticae *and* T. cinnabarinus; *percentages surviving of at least 30 specimens*

Time	+ 3° C		— 5° C		— 16° C		— 22° C	
kept at	*T. urt.*	*T. cinn.*	*T. urt.*	*T. cinn.*	*T. urt.*	*T. cinn.*	*T. urt.*	*T. cinn.*
1 day	100	100	100	95	100	70	100	35
5 days	90	94	95	100	95	4	100	0
21 days	82	86	100	96				
35 days	100	80	100	56				
49 days	95	75	95	15				
56 days	90	84	96	12				

This difference in cold-resistance is the reason why in springtime *T. cinnabarinus* was never found in the field. We could observe in the beginning of the winter that the mites that had clustered together on decaying leaves in the field perished, especially by drowning in the cold water of melting snow. This was very evident in the winter of 1959 - 1960.

CROSSING EXPERIMENTS WITH T. URTICAE AND T. CINNABARINUS

Female deutonymphs and teleiochrysali, and adult males from different host plants were isolated and placed on leaf discs as described before.

Three series of experiments were done, viz. (1) crossings between *urticae*-populations of different origin; (2) crossings between *cinnabarinus*-populations of different origin; (3) crossings between the populations sub (1) and (2). All crossings were carried out reciprocally, the number of pairs of crossings being five as a rule. The observations included the mating of the parent animals, the fertility of the offspring (F1) and the development of the F2 generation.

TABLE VII

Mutual crossings between mite populations of T. urticae *from several hostplants.*

	Rose	Bean	Tomato	*Sambucus*	*Urtica*	*Althaea*	*Chelidonium*	*Euonymus*
Rose	+							
Bean	+	+						
Tomato	+		+					
Sambucus	+	+	+	+				
Urtica		+	+	+	+			
Althaea		+	+	+	+	+		
Chelidonium	+		+	+			+	
Euonymus	+		+	+	+		+	+

1. Mutual crossings between *T. urticae* succeeded in all circumstances and combinations, as is shown in Table VII. If a favourable development, i.e. F2, was noticed, a + sign was inserted in the Table.

Reciprocal crossings were also made between populations originating from places in different countries of North West Europe, namely Switzerland (Basel) and Germany (Bentheim):

			Number of reciprocal crossings
French bean (Basel)	×	*Chelidonium* (Aalsmeer)	4
French bean (Basel)	×	Rose (Aalsmeer)	17
Sambucus (Bentheim)	×	Rose (Aalsmeer)	4
Sambucus (Bentheim)	×	*Buddleya* (Aalsmeer)	4

The result of these crossings was the same as before: in all cases the mites mated freely and a large progeny was formed.

Interbreeding experiments gave no indication that more than one species of *Tetranychus urticae* was involved in these experiments.

2. The populations *Cinnabarinus* 1 and *Cinnabarinus* 2 (see Page 148) were crossed. Six pairs of reciprocal experiments showed, however, in all cases a fertile and flourishing F2.

Crossings were made also between *cinnabarinus*-populations, three of them originating from Germany (Frankfurt a/Main, Xanthen, and Kleve), and one from Belgium (Aalst), with the strain *Cinnabarinus* 1 from Aalsmeer. The number of pairs of reciprocal crossings were:

Carnation	(Frankfurt a/Main)	×	Carnation =Cinnabarinus 1	(Aalsmeer)	6
Rosa	(Frankfurt a/Main)	×	,,	,,	6
Carnation	(Xanthen)	×	,,	,,	4
Carnation	(Kleve)	×	,,	,,	4
Carnations	(Aalst)	×	,,	,,	6
Carnation	(Xanthen)	×	Rosa	(Frankfurt a/Main)	4

Here also, the mating and offspring in all experiments succeeded, and a large F2 generation was obtained. No evidence about the existence of more than one species in the *cinnabarinus* complex, founded on interbreeding, was found.

3. Crossings were made between *T. urticae* populations of different origin and those of *T. cinnabarinus*. In total 35 crossings were made in the following combinations:

Urticae		*Cinnabarinus*	Nr. reciprocal cross.
Rosa (Aalsmeer)	×	Cinnabarinus 1 (Aalsmeer)	5
French bean (Wageningen)	×	,,	5
Tomato (Wageningen)	×	,,	5
Sambucus (Aalsmeer)	×	,,	5
Chelidonium (Wageningen)	×	,,	5
Euonymus (Wageningen)	×	,,	5
Althaea (Wageningen)	×	,,	5

Mating and oviposition succeeded in all cases, but a hybrid was obtained in 31 crossings which either did not lay eggs at all, or only sterile eggs were laid.

Four crossings with mites from *Chelidonium, Euonymus* and *Althaea* resulted in a fertile hybrid generation, very small in number, and some specimens of an F2 generation were obtained which were all sterile. Some of the crossings are shown in detail in Fig. 9.

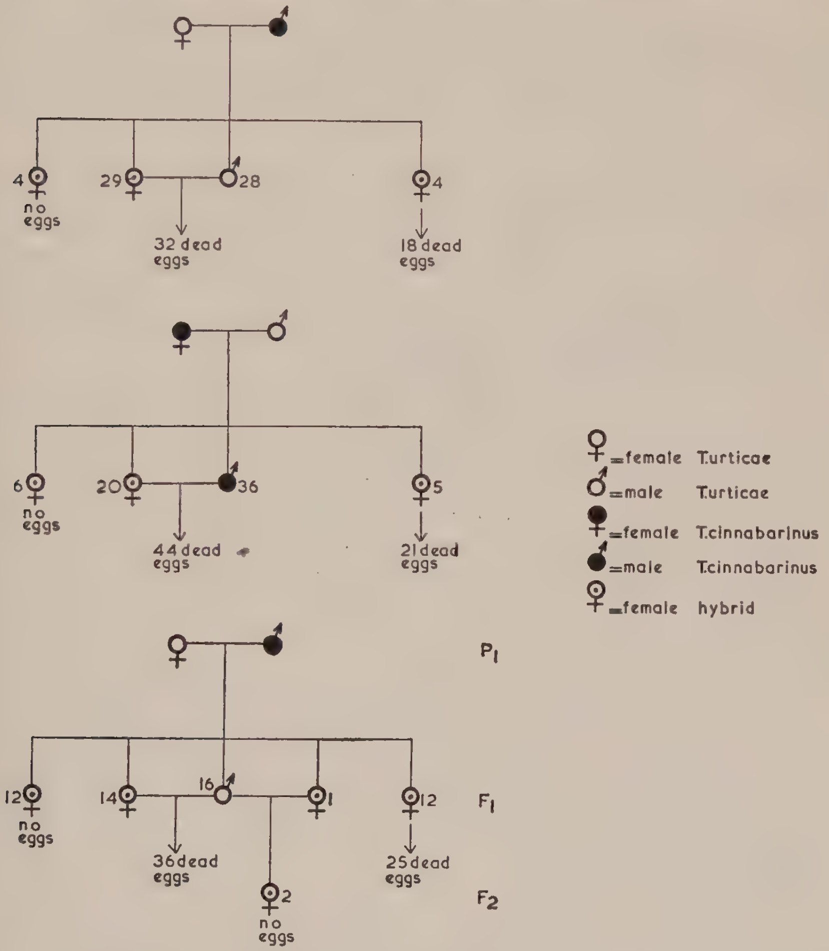

Fig. 9. Crossings between *T. urticae* and *T. cinnabarinus.*

The crossing experiments, therefore, corroborated our conclusion that two true species can be distinguished.

DISCUSSION

In this work differences between *T. urticae* and *T. cinnabarinus* were found in morphology, host plant preference, habit of hibernation, including cold resistance and diapause, and also in crossing experiments. All results point to the fact, that two different species are involved. However, we failed to find differences between populations of the *T. cinnabarinus* complex of different origin, and the same was the case with the *T. urticae (sensu stricto)* complex. The difference in behaviour to cold makes apparent that *T. cinnabarinus* originates from the Mediterranean region, and is not indigenous to N.W. Europe.

BOUDREAUX (1956) gives a key to the species *T. cinnabarinus, T. telarius* and *T. lobosus.* The females are distinguished by the form of the lobes of the striae, which according to our experience is difficult to interpret, as variation is very large, and overlapping of characters in both species is frequent. The form of the aedeagus seems to be a more reliable feature but interpretation is hampered by (1) the position of the specimen on the slide, (2) differences in optical equipment used.

The lack of reliability of morphological characters, however, is very well illustrated by BOUDREAUX (1959) in his work on the taxonomic value of extra setae which happened to be influenced by a virus-like disease and which was not hereditary at all. *T. multisetis* (MCGREGOR 1950), described mainly on this character, cannot be maintained as a valid species, and the same must be said about the description of *T. urticae* forma *dianthica* (DOSSE 1952). Females with extra setae were found in the Netherlands also (Fig. 6).

A small difference in the form of the aedeagus was the reason that DILLON (1958) expressed the opinion that two different species of *T. cinnabarinus* should exist, one in England and one in Germany. However, both forms of aedeagus are found in Aalsmeer, and the mites cross freely.

In his first paper on *T. cinnabarinus,* BOUDREAUX (1956) called attention to the fact that this species had a diapause form which becomes apparent at low temperatures. Later-on (1958) the same author, however, writes that this species is always able to oviposit at favourable temperatures, and a real diapause does not exist. This agrees well with our observations. We see no reason why HUSSEY & PARR (1958) should maintain their opinion that the 'reddish-brown form' found in England on carnations is not the same as Boudreaux's *T. cinnabarinus*[1]).

In the past ten years millions and millions of cuttings of carnations have been exchanged between many countries of the continent of Europe and the British Isles. *T. cinnabarinus* will have been exchanged also, as conditions in glasshouses in different countries are much the same, and it would be amazing if large differences in spider mites on carnations exist.

The results of our crossing experiments are in agreement with those of other authors (BOUDREAUX 1956; HUSSEY & PARR 1958; DILLON 1958).

As several authors have said before (MCGREGOR & NEWCOMER 1928; KEH 1952), biological features should be ranked first if descriptions of new species of spider mites are given. Of these features, lack of interbreeding is the most important. However, here also the differences do not always seem clear. Sometimes only a slight incompatibility between two strains can be found, demonstrated by a slightly smaller number of progeny, or a higher number of males; another time the second generation is wholly sterile, and the existence of two species is apparent.

CONCLUSION

In the Netherlands *T. urticae* is the most common spider mite, both in the field and in greenhouses. This species is well adapted to the climate of North West Europe and very cold resistant. In summer time this species may develop as a pest of several crops, indoors as well as outdoors, with the exception of carnation. No indication is found of special biological races on specific host plants. The possibilities of *T. urticae* for developing to harmful numbers are very good.

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[1]) Recently (PARR & HUSSEY, 1960) the authors revised their view, and came to same conclusions, independent of us. (Note added to the proof).

T. cinnabarinus is found in the Netherlands in greenhouses and their immediate surroundings only. The carmine colour of the adult summer females makes it easy for the grower to distinguish. This species does often damage on carnation. As it is not able to hibernate in the field, invasions of spider mites from outside in early spring are not to be feared by the carnation grower. By cleaning the glasshouses during winter time, and using cuttings which are cleared of mites, it will be possible to the carnation grower to cultivate his crop without spider mites, and to keep it free by preventive measures only.

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ZUSAMMENFASSUNG

UNTERSUCHUNGEN ÜBER DEN TETRANYCHUS URTICAE- *KOMPLEX IN NORDWEST-EUROPA (ACARI : TETRANYCHIDAE)*

Untersuchungen der Morphologie, der Wirtspflanzenwahl, der Überwinterungsweise und Kreuzingsexperimente haben gezeigt, daß in Gewächshäusern Hollands zwei Spinnmilben-Arten auftreten, nämlich *Tetranychus urticae* Koch und *T. cinnabarinus* Boisd. Darüberhinaus wurde Material von Spinnmilben je einer Lokalität in Deutschland, der Schweiz und Belgien verwendet.

T. urticae ist meistens grün gefärbt, lebt an einem großen Wirtspflanzenkreis, kann aber an Nelken (*Dianthus caryophyllus* L.) keine Populationen bilden. Diese Art geht unter dem Einfluß verschiedener biologischer Faktoren in Diapause. Während dieser Periode ist sie sehr kälteresistent. Der Winter wird an geschützten Orten verbracht. Kreuzungen zwischen Populationen verschiedener Herkunft ergaben stets eine normale Nachkommenschaft. Zuchtexperimente mit diesen Populationen auf verschiedenen Wirtspflanzen ergaben keinen Hinweis für das Bestehen von biologischen Rassen bei *T. urticae.*

T. cinnabarinus ist karminrot gefärbt und wird am häufigsten auf Nelken gefunden, obwohl in Laboratoriumsversuchen die Entwicklung an Buschbohnen (*Phaseolus vulgaris* L.) schneller verläuft. Diese Art tritt überhaupt nicht in Diapause ein, bleibt den Winter über an den Blättern und ist gegenüber Kältebedingungen entschieden weniger resistent als *T. urticae.* Kreuzungen zwischen Populationen verschiedener Herkunft ergaben immer eine normale Nachkommenschaft.

Das Ausbleiben von Bastardierungen, das in Kreuzungsexperimenten zwischen den beiden Arten gefunden wurde, erbrachte den strengsten Nachweis, daß in den Gewächshäusern zwei verschiedene Arten vorkommen.

Da *T. cinnabarinus* hauptsächlich auf Gewächshäuser mit Nelken beschränkt ist und *T. urticae* sich andererseits an diesen Pflanzen nicht vermehrt, haben es die Nelkenanbauer lediglich mit *T. cinnabarinus* zu tun, und es ergibt sich eine Möglichkeit, Nelken frei von Spinnmilbenbefall zu halten.

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ÜBER DIE URSACHEN DER UNTERSCHIEDLICHEN RESISTENZ VON *VICIA FABA* L. GEGENÜBER DER BOHNENBLATTLAUS, *APHIS (DORALIS) FABAE* SCOP.

VI. Die Resistenzerscheinungen an Reisern reziproker Pfropfungen anfälliger und resistenter Ackerbohnen[1]).

VON

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Das Präferenz-Nonpräferenzverhalten sowie die antibiotisch beeinflußte Höhe der Reproduktionsrate von *Aphis fabae* gegenüber Ackerbohnensorten *(Vicia faba)* änderten sich nicht, wenn eine resistente Sorte (Rastatter) auf eine anfällige (Schlanstedter) gepfropft wurde oder umgekehrt. Verhalten und Vermehrung der Blattläuse richteten sich stets nach dem Sortencharakter des Reises, unabhängig von dessen Unterlage.

Die relativ weitgehende Resistenz der Rastatter Ackerbohnen gegenüber der Besiedlung mit der Schwarzen Bohnenlaus, *Aphis* (*Doralis*) *fabae* Scop., beruht, wie frühere Untersuchungen gezeigt haben (H. J. MÜLLER 1953, 1958), auf zwei verschiedenen Mechanismen: auf einem Präferenzverhalten der geschlechtsreifen Jungfern, die andere Ackerbohnensorten, speziell die Schlanstedter (S), weit häufiger besiedeln als die Rastatter (R) (im Freien 70—80% der Initialkolonien auf S), und auf antibiotischen Wirkungen der Rastatter, auf denen unter sonst gleichen Bedingungen Reproduktionsrate, Entwicklungs- und Lebensdauer der Aphiden gegenüber anderen Sorten erheblich verringert sind.

Auf der Suche nach den Ursachen dieser Erscheinungen, speziell nach einer stofflichen Grundlage der Resistenz, wurden vom zweitgenannten Autor wechselseitige Pfropfungen junger Rastatter und Schlanstedter Ackerbohnen hergestellt. Bei der Durchführung von Wahl- und Aufzuchtversuchen mit Bohnenläusen auf den Pfropfungen mußte sich im Vergleich mit nichtgepfropften Kontrollen zeigen, ob die Resistenzerscheinungen am Reis, also vor allem Wirtswahl- und Reproduktionsrate, von der Unterlage beeinflußt werden oder nicht.

Im Verlauf der Experimente erhielten wir Kenntnis von ähnlichen Untersuchungen van HARVEY & HACKEROTT (1958) mit *Therioaphis maculata* (Buckton) an Luzernepfropfungen, welche diese Frage im gleichen Sinne beantworten wie unsere Ergebnisse (s.u.).

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[1]) Quedlinburger Beiträge zur Züchtungsforschung Nr. 44.

HERSTELLUNG DER PFROPFUNGEN

Die beiden Sorten (R und S) waren wechselweise so aufeinanderzupfropfen, daß einmal R als Unterlage und S als Reis diente, das andere Mal umgekehrt. Dabei kam eine von den üblichen Pfropfverfahren etwas abweichende Methode zur Anwendung, deren Vorteil darin besteht, daß eine in die beiderseitigen Markhohlräume eingeführte Glaskapillare beide Pfropfpartner gut aufeinanderpaßt, verbindet und stützt.

Drei Wochen alte, einzeln im Gewächshaus auf 12—20 cm Höhe herangezogene Jungpflanzen werden mit einer scharfen Rasierklinge unterhalb des ersten oder zwischen erstem und zweitem Primärblatt schräg durchschnitten. Reis (z.B. der obere, abgeschnittene Teil einer R-Pflanze) und Unterlage (im Beispiel der stehengebliebene Stumpf einer S-Pflanze) werden sodann dadurch verbunden, daß eine in der Größe passende Glaskapillare mit ihrer unteren Hälfte in den Markraum der Unterlage eingeführt und dann das Reis auf die frei nach oben herausragende Hälfte aufgeschoben wird (Abb. 1), bis sich die Schnittflächen der Pfropfpartner berühren. Damit die Glaskapillare in beiden Teilen festsitzen und dadurch der Pfropfung den erforderlichen Halt geben kann, muß ihr Durchmesser auf die Weite der zu verbindenden Markhöhlen abgestimmt sein. Praktisch hält man schon vorher ausgezogene Kapillaren verschiedener Länge und Stärke in größerer Anzahl bereit, so daß für jede Pfropfung nur noch die passende ausgewählt zu werden braucht. Bei Partnern, die im Reis eine größere Markhöhle

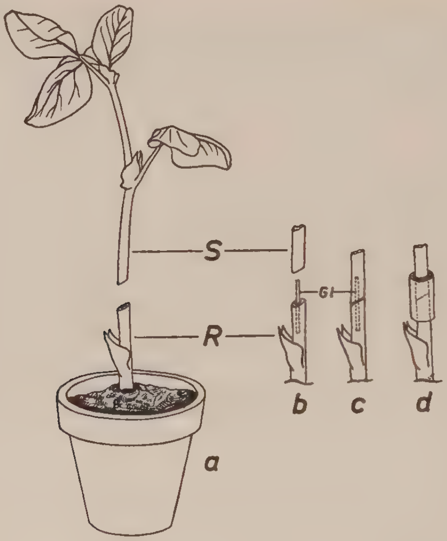

Abb. 1. Schema der Pfropftechnik am Beispiel einer S-Pfropfung auf eine R-Unterlage (a); b. untere Hälfte der Glaskapillare (Gl) in die Markhöhle der Unterlage (R) eingeführt; c. Markhöhle des Reises (S) auf die freie obere Hälfte der Glaskapillare aufgeschoben; d. Wickelung der Pfropfstelle mit Globoplast.

aufweisen als in der Unterlage — oder umgekehrt —, verwendet man Kapillaren, die an ihren Enden verschieden große Querschnitte besitzen.

Auch die Schnittflächen von Reis und Unterlage müssen genau zueinander passen. Da aber die Stengeldurchmesser auch bei gleichaltrigen Pflanzen oft verschieden groß sind, wird in solchen Fällen am besten Reis oder Unterlage nachträglich noch so weit zurückgeschnitten, daß beide annähernd gleich große Stengelquerschnittflächen aufweisen.

Zuletzt wird die Pfropfstelle fest mit einem 8—10 cm langen und 1 cm breiten Klebstreifen umwickelt, wie er für medizinische Verbände benutzt wird (Globoplast), und anschließend die Pflanze an einem Glasstab hochgebunden.

Die so gepfropften Pflanzen wurden für ca. 3 Wochen in einer geschlossenen Gewächshauskabine von 22 m^3 aufgestellt. Dort herrschten 20—25° C und während der ersten 10 Tage 100% rel. Luftfeuchtigkeit; danach jeden weiteren Tag 10% weniger, also am 11. Tage 90%, am 12. Tage 80% usw. Die gewünschte Luftfeuchtigkeit erzeugte ein Defensor (Modell 3001 der Firma Barth & Stöcklein, München), der von einem Hygrostaten über ein Relais gesteuert wurde. Vom 16. Tage an wurden die Kabinenfenster geöffnet, um die jungen Pfropfpflanzen wieder an natürliche Außenluft zu gewöhnen. Absterbende Pflanzen wurden bei den täglichen Kontrollen entfernt, so daß nur die tatsächlich zusammengewachsenen übrigblieben.

Die Ergebnisse waren recht unterschiedlich. Anfangs wuchsen nur etwa 20% aller gepfropften Pflanzen zusammen, während die übrigen meist schon in den ersten 8 Tagen abstarben. Die Ursache hierfür schienen Gewebeschädigungen aller Art zu sein, die größtenteils beim Pfropfen entstanden waren. Als weniger schädlich erwies sich der mehr oder weniger starke Pilzbefall, der während der gleichen Zeit an fast allen Pflanzen zu beobachten war. Er beschleunigte lediglich das Absterben der bereits anderweitig geschädigten Pflanzen. Durch Verbesserung der Pfropftechnik ließen sich die Verluste jedoch schließlich so weit verringern, daß durchschnittlich 70—75% der gepfropften Pflanzen verwuchsen und überlebten.

WIRTSWAHLVERSUCHE AN PFROPFUNGEN (PRÄFERENZ)

Methodik

In einer ersten Versuchsserie sollte zunächst das Wahlverhalten von Bohnenläusen gegenüber Pfropfpflanzen ($\frac{S}{R}$ oder $\frac{R}{S}$) im Vergleich mit ungepfropften S- bzw. R-Pflanzen geprüft werden. Für die Durchführung dieser Wahlversuche erwies sich die sogenannte Pertelarmethode[1]), wie sie MÜLLER (1954) für Zikaden beschrieben hat, in entsprechend abgewandelter Form als besonders geeignet.

Die Aphiden werden dabei einzeln in Glaszylindern von 5 cm Höhe und 4 cm Durchmesser gehalten, die auf beiden Seiten mit glattem Perlongswebe bespannt sind, zwischen dessen Maschen die Läuse ihr Rostrum mühelos hindurchstecken und in außen aufgelegte Pflanzengewebe versenken können. Die Perlonbespannung der unteren Zylinderöffnung wird nur durch einen Gummiring gehalten, so daß sich die Blattläuse hier leicht einsetzen bzw. entnehmen lassen. Die obere ist dagegen am Rand festgeklebt und wird von außen mit einem Blatt der Nährpflanze bedeckt. Der konvex gewölbte Boden und das Gewicht eines wassergefüllten Glasschälchens pressen das Blatt gleichmäßig gegen die Maschen des Gewebes, so daß es für die Aphiden — bis auf einen peripheren Bezirk am Rande — überall gut zugänglich ist.

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[1]) Beiderseits mit Gazegewebe bespannte Käfige (Glaszylinder), bei denen Sauginsekten ihre Nahrungsquelle nur erreichen können, wenn sie ihren Rüssel durch die Maschen des Gewebes (lat.: *per telam*) hindurchstecken.

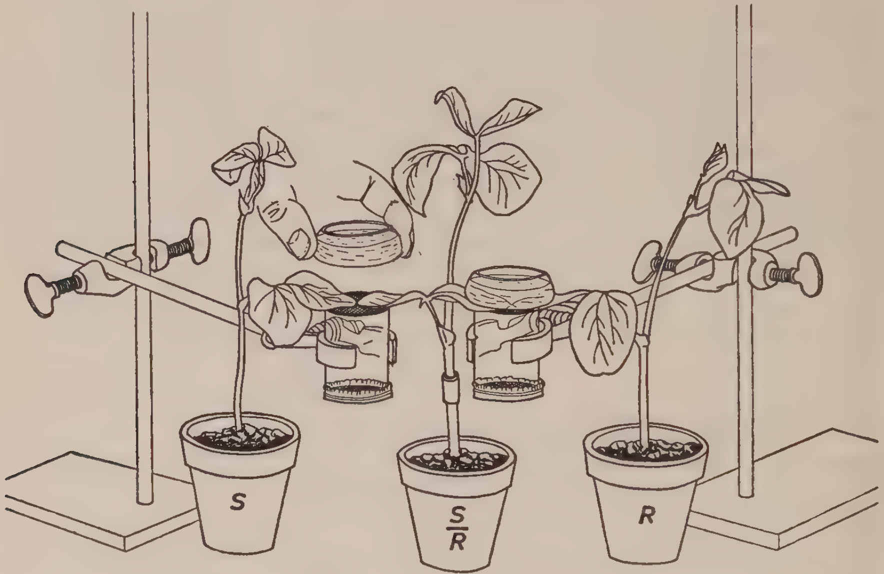

Abb. 2. Anordnung der Pertelar-Wahlkäfige für die simultane Prüfung eines S-Reises mit einer S-Pflanze (links) und mit einer R-Pflanze (rechts). Links soll das wassergefüllte Gewichtsglas eben auf die obere Perlonbespannung aufgesetzt werden, auf der die Spitzen der zur Wahl angebotenen Blätter bereits aufliegen.

Für die Wahlversuche wurden die Zylinder senkrecht so in Stativklammern gespannt, daß sie in Höhe der zu prüfenden Blätter kamen (Abb. 2). Auf die obere (festgeklebte) Perlonbespannung wurden dann ein Blatt vom Reis einer Pfropfpflanze ($\frac{S}{R}$ oder $\frac{R}{S}$) und eins von einer daneben gestellten (nichtgepfropften) Vergleichspflanze (R oder S) jeweils mit der Unterseite so unter das Gewichtsglas gelegt, daß sich ihre Spitzen in der Mitte eben berührten und ihre Hauptadern zugleich eine Gerade bildeten. Der zu prüfenden Laus standen dadurch jeweils etwa gleich große Flächen der beiden Blätter zur Wahl. In der Regel wurden nur Primärblätter gleicher Stellung am Sproß, gleicher Größe und damit annähernd gleichen Alters verwendet. Da sich das linke und das rechte Fiederblatt der Pfropfpflanze gleichzeitig auf zwei verschiedene Wahlkäfige legen lassen, konnte das Reis jeweils in einem Versuchsgang mit einer R- und mit einer S-Pflanze verglichen werden.

Sämtliche Versuche liefen in der Zeit von Mitte April bis Ende Juni 1959 in einem behelfsmäßig klimatisierten Gewächshaus (bei tunlichst 20° C), anfangs mit 12, später mit 24 Käfigen. Die Pfropfungen waren dann jeweils ca. 4 Wochen alt. Als Versuchstiere dienten ungeflügelte, virginogene Jungfern von *Aphis fabae*, die auf Spinatblättern (abgeschnitten und in Wasser eingefrischt) herangezogen wurden, damit ihr Wahlverhalten nicht schon vorher durch Ackerbohnen beeinflußt werden konnte.

Zwischen 7 und 8 Uhr morgens wurde jeder Wahlkäfig mit je einer Imago besetzt, die bis dahin noch keine Larven geboren hatte. Um zu erfahren, ob jede wählende Laus auch wirklich mit beiden zur Wahl stehenden Blättern in Berührung gekommen war, mußte sie zunächst so lange ununterbrochen beobachtet werden, bis sie bei ihrem anfänglichen Umherwandern auf jedem der Blätter wenigstens einmal ihre Rüsselspitze aufgesetzt hatte. Anderenfalls wurde ihre spätere Ansiedlung nicht als Wahlakt bewertet. Die weiteren Kontrollen erfolgten dann bis 13 Uhr in Abständen von 10 min- und von 13—17 Uhr in 20 min-Intervallen, danach noch je einmal um 19 und 21 Uhr sowie um 7 Uhr am folgenden Morgen bei der Beendigung jeder Einzelversuchs vor der Säuberung des Käfigs. Jede Kontrolle hatte zu ermitteln: 1. ob und an welchem der gebotenen Blätter sich die betreffende Laus festgesetzt und 2. ob und wie viele Junglarven sie hier geboren hatte. Jede Versuchspflanze wurde an 14 aufeinanderfolgenden Tagen für die Wahlversuche benutzt und dann durch eine neue ersetzt.

Entgegen Wahlversuchen mit anderer Fragestellung wurde hier als entscheidendes Kriterium die endgültige Ansiedlung und Bildung einer Initialkolonie angesehen. Deshalb galt als gewählt nur das Blatt, auf dem die zu prüfende Bohnenlaus wenigstens während der letzten 18 (von den insgesamt 24) Stunden des Versuchs ununterbrochen an der gleichen Stelle festgesessen und mindestens eine Junglarve abgesetzt hatte. Während der ersten Stunden laufen die Läuse meist noch unruhig umher und verlassen auch die zunächst und selbst für längere Zeit besiedelten Plätze häufig noch wieder, oft ohne Larven produziert zu haben. Es stand aber in den vorliegenden Untersuchungen nicht zur Diskussion, wie oft eine bestimmte Laus unter den gegebenen Umständen ihre Pflanze wechselte und erneut wählte, obwohl unveröffentlichte, mit ähnlicher Methodik durchgeführte Untersuchungen über den Wahlakt selbst zeigen, daß bei der Benutzung des Ortes des allerersten, u.U. flüchtigen Festsetzens oder des Geburtsortes der ersten Larve als Wahlkriterium ganz ähnliche Ergebnisse auftreten (s.u.). Es wurden aber auch alle Fälle als „nicht gewählt" ausgeschieden, in denen die Blattlaus während des Versuches starb, ihren Platz während der letzten 18 Stunden noch einmal wechselte oder keine Junglarven dort abgesetzt hatte. Insgesamt wurden 740 *Aphis fabae*-Jungfern zur Wahl angesetzt und zwar: 202 zur Wahl zwischen dem Blatt eines S-Reises auf R- Unterlage ($\frac{S}{R}$) und dem Blatt einer normalen S-Pflanze (S) = Wahlkombination $\frac{S}{R}$: S, und in entsprechender Weise 144 zur Wahl $\frac{S}{R}$: R, 146 zur Wahl $\frac{R}{S}$: S und 248 zur Wahl $\frac{R}{S}$: R (siehe Tab. I, 1. und 2. Spalte).

Ergebnisse der Blattwahlen

Betrachtet man in jeder Kombination zunächst nur den Prozentsatz der Läuse, die sich im Sinne der oben genannten Kriterien während des Versuchs überhaupt angesiedelt hatten, ohne die Wahl selbst zu berücksichtigen, so ergeben sich schon außerordentlich eindrucksvolle Beziehungen (Tab. I, 3. Spalte). Die meisten Initialkolonien (90,6%) wurden nämlich dort gebildet, wo den wählenden Läusen nur S-Blätter, je eins vom Pfropfreis und eins von der Kontrollpflanze, zur Verfügung standen. Die meisten Nichtwähler (51,2%) fanden sich dagegen, wenn nur R-Blätter vorhanden waren (R-Reis und R-Kontrolle). In den beiden Kombinationen, in denen jeweils ein R-Blatt und ein S-Blatt (entweder als Reis oder als Kontrolle oder vice versa) angeboten wurden, hält sich die Anzahl der Nichtwählenden etwa in gleicher Höhe: 21,5% bei $\frac{S}{R}$: R und 23,3% bei $\frac{R}{S}$: S. Es spiegelt sich bereits in diesen Verhältnissen ganz eindeutig die aus früheren Versuchen bekannte Bevorzugung der S-Pflanzen vor R-Pflanzen wieder: je mehr S-Blätter zur Verfügung stehen, um so besser ist die Ansiedlung: am besten ist sie, wenn zwei S-Blätter, am schlechtesten, wenn stattdessen nur zwei R-Blätter gereicht werden. Wahlkäfige mit einem S-Blatt liegen bezüglich der Ansiedlungsrate ziemlich genau in der Mitte zwischen diesen Werten.

Schon diese Relationen beweisen, daß die Unterlage offensichtlich keine Wirkung auf das Reis auszuüben vermag, welche die Wahl der Läuse beeinflussen könnte. Für die wählende Laus ist der Sortencharakter des Blattes entscheidend, ganz gleich, ob es auf einer fremden Unterlage sitzt oder nicht.

TABELLE I

Ergebnisse der Wahlversuche mit ungeflügelten Virginoparen von Aphis fabae *an Blättern gepfropfter und normaler Ackerbohnen (S = Schlanstedter, R = Rastatter)*

Wahlkombination zwischen entsprechenden Blättern des *Pfropfreises* (oben) und der *Normalpflanze*	n Anzahl der angesetzten Mutterläuse	Prozentsatz der Nichtwählenden und Ausgeschiedenen	Prozentverhältnis zwischen der Ansiedlung auf dem Blatt des *Pfropfreises* und der *Normalpflanze*	
$\frac{S}{R}$: S	202	9,4	48,1 : 51,9	S:S ≌ 1:1
$\frac{S}{R}$: R	144	21,5	79,7 : 20,3	S:R ≌ 4:1
$\frac{R}{S}$: S	146	23,3	27,9 : 72,1	R:S ≌ 1:2,6
$\frac{R}{S}$: R	248	51,2	47,0 : 53,0	R:R ≌ 1:1

Das bestätigt sich, wenn die Ergebnisse der eigentlichen Wahl in den vier geprüften Kombinationen miteinander verglichen werden (Tab. I, Spalte 4 und 5). Standen den wählenden Läusen gleichnamige Blätter zur Verfügung, d.h. gehörten Pfropfreis und Kontrollpflanze der gleichen Sorte an, so wurden sie beide annähernd gleich häufig zur Gründung einer Initialkolonie besiedelt, ganz gleich, ob es sich um zwei Rastatter (Kombination $\frac{R}{S}$: R) oder um zwei Schlanstedter (Kombination $\frac{S}{R}$: S) Blätter handelte. Das könnte nicht der Fall sein, wenn die Unterlage die Eigenschaften des Reises in seiner Wahlqualität für die Bohnenläuse veränderte. Allerdings ist die Häufigkeitsverteilung nie 1 : 1, sondern stets etwas zugunsten der ungepfropften Normalpflanze verschoben (Tab. I). Die Ursache davon wird weiter unten zu diskutieren sein; sie beruht jedoch unter keinen Umständen auf einer Wechselwirkung zwischen Unterlage und Reis. Hatten die Läuse aber die Wahl zwischen einem R- und einem S-Blatt, so bevorzugten sie stets die S-Blätter um ein Vielfaches, völlig unabhängig davon, ob das S-Blatt einem Pfropfreis auf einer R-Unterlage oder einer normalen S-Vollpflanze angehörte. So wählten in der Kombination $\frac{S}{R}$: R fast 80% die Pfropfung, weil sie ein S-Reis hatte, in der Kombination $\frac{R}{S}$: S aber 72,1% die Normalpflanze, denn als Unterlage wirkt S praktisch als nichtexistent gegenüber der S-Vollpflanze. Umgekehrt wurden R-Blätter auch dann abgelehnt, wenn sie von einer S-Unterlage getragen wurden.

DIE ANSIEDLUNGS- UND REPRODUKTIONSRATE (ANTIBIOSISWIRKUNG) AUF DEN PFROPFREISERN

Methodik

Im Anschluß an die Wahlversuche wurden die (inzwischen noch mehr herangewachsenen und etwas vergeilten) Pfropfpartner zur Prüfung von Größe und Dauer der sogenannten Erstgeborenen-Produktion in folgender Weise vorbereitet: Der Gipfel des Reises wurde bis auf die Höhe des jüngsten, aber voll entfalteten Primärblattes zurückgeschnitten und zugleich alle unter diesem stehenden (älteren) Blätter des Reises sowie der Unterlage entfernt, der Boden zur besseren Übersicht mit einer Schicht weißen Sandes bedeckt und der Topf auf einem umgekehrten Untersatz in eine flache Schale mit Wasser gesetzt (Abb. 3). Etwa nachträglich von Unterlage oder Reis austreibende Ersatzknospen wurden stets sorgfältig zurückgeschnitten und die durch das Abschneiden des Gipfels entstandene Öffnung des Markhohlraumes mit einem Wattepfropf verschlossen, um das Einwandern

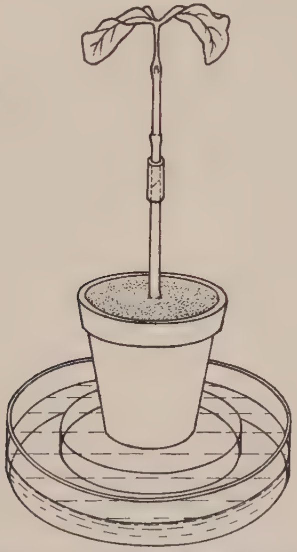

Abb. 3. Pfropfpflanze zur Prüfung des Antibiosiseffektes am Reis (Reproduktionsrate) vorbereitet.

und Ansiedeln der Läuse in diesem zu verhindern. Die Läuse lebten ausschließlich auf dem Reis, meist auf der Unterseite der Blattspreite, manchmal auch am Stengel.

Umfang und Dauer der Erstgeborenen-Generationen wurden in der schon früher (Müller 1958) angegebenen Weise ermittelt; d.h. 1. die Anzahl der Töchter festgestellt, welche jede (einzeln auf das Reis gesetzte) ungeflügelte Mutterlaus von der Geburt bis zur Reife ihrer erstgeborenen Tochter produzierte, und 2. der dazu benötigte Zeitabschnitt in Tagen gemessen. Tochterfamilien, bei denen die Mutter bereits vorher starb oder abwanderte, wurden ausgeschieden und besonders gezählt, ebenso diejenigen Jungfern, welche sich aus irgendeinem Grunde überhaupt nicht auf der Pflanze ansiedelten, verunglückten oder abwanderten, bevor sie Larven auf ihr abgesetzt hatten. Der Anteil der letzteren ist auf R- und S-Reisern verschieden groß und spiegelt das Wirtswahlverhalten wieder (s.u.).

Abgesehen von einem Vorversuch mit relativ wenigen Pflanzen standen für die Reproduktionsprüfungen 16 Pfropfungen von Rastatter auf Schlanstedter $\left(\frac{R}{S}\right)$ und 16 Pfropfungen von Schlanstedter auf Rastatter $\left(\frac{S}{R}\right)$ sowie je 16 etwa gleichaltrige normale Rastatter und Schlanstedter Jungpflanzen zur Verfügung. Da sie mehrere Wochen (Mai-Juni 1959) gesund und wüchsig blieben, konnten nacheinander durchschnittlich 4—6 Erstgeborenen-Nachkommenschaften auf ihnen aufgezogen werden. Die Pflanzen standen frei in einem nur bei starker Besonnung schattiertem Gewächshaus bei Temperaturen, die nach Möglichkeit bei 20° C gehalten wurden (nachts bis 15°, bei starker Insolation mittags über 30° C). Die Entwicklung der Blattläuse wurde täglich — zur Zeit der herannahenden Reife der Erstgeborenen mehrmals — kontrolliert.

Ergebnisse

Obwohl sich der zweite Teil der vorliegenden Untersuchungen vorwiegend mit der Reaktion der Reproduktionsrate der Bohnenläuse auf die antibiotische Komponente der Pfropfungen befaßt, läßt das dabei gewonnene Material zusätzlich auch noch einige weitere Aussagen über die Präferenzkomponente zu, und zwar — als Ergänzung und Bestätigung zu der im ersten Teil geschilderten Wahl des endgültigen Ansiedlungsortes — vorwiegend über den primären Wahlakt selbst. Im Gegensatz zu den Käfigwahlversuchen waren die Läuse hier völlig frei und hatten nicht zwischen zwei Blättern zu wählen, sondern nur über Annahme oder Ablehnung je einer Pflanze zu entscheiden, die sie jederzeit wieder verlassen konnten.

Um auf jeder der 16 Pfropfungen und Kontrollpflanzen nacheinander eine möglichst gleich große Anzahl von Erstgeborenen-Generationen zu erzeugen, mußte nämlich je nach Art des Reises bzw. der Kontrollpflanze eine verschieden große Anzahl von jungen Mutterläusen angesetzt werden (Tab. II), da ein wechselnder Prozentsatz derselben besonders die R-Pflanzen hartnäckig wieder verließ, ohne Larven abgesetzt zu haben. Sowohl die Anzahl der zur wiederholten gleichmäßigen Besiedlung notwendigen Mutterläuse wie auch der Prozentsatz derjenigen unter ihnen, die sich wirklich ansiedelten und Initialkolonien begründeten, kann deshalb als Ausdruck und Maßstab für das Präferenzverhalten gegenüber den Pfropfungen und Kontrollen gewertet werden.

TABELLE II

Wirtswahlverhalten der virginoparen Mütterläuse in den erstgeborenen Zuchten (Einzelheiten im Text).

Pfropfpartner und Kontrollpflanze	$\frac{R}{S}$	R	$\frac{S}{R}$	S
Anzahl der auf je 16 Pflanzen nacheinander angesetzten Jungfern	272	243	155	160
Prozentsatz der davon Initialkolonien gründenden Jungfern	46,0	56,8	60,3	74,1
Prozentsatz der bis zur Reife der erstgeborenen Tochter in der Initialkolonie seßhaft bleibenden Jungfern	17,7	21,8	43,9	51,0

Betrachtet man zunächst die Kontrollen, (Tab. II, Zeile 2), so zeigt sich das von früheren, z.T. unveröffentlichten Untersuchungen gewohnte Bild: fast ¾ aller auf S angesetzten Läuse nimmt die Pflanzen an, während auf R nur reichlich die Hälfte dazu bereit sind. Infolgedessen war zur Besiedlung der R-Kontrollen weit mehr als das Anderthalbfache der für S benötigten Jungfern erforderlich. Auf den Pfropfungen liegen die Verhältnisse ganz ähnlich; sie sind nur parallel zur negativen Seite hin verschoben, d.h. auf dem S-Reis $\left(\frac{S}{R}\right)$ siedeln sich mit 60% etwa $^1/_3$ mehr Jungfern an als auf dem R-Reis $\left(\frac{R}{S}\right)$ (46%), aber auf $\frac{S}{R}$ weniger als auf S und auf $\frac{R}{S}$ weniger als auf R.

Ein großer Teil der Mutterläuse verläßt jedoch die Pflanzen auch noch, nachdem schon einige oder mehrere Junglarven abgesetzt worden sind (und einige andere vorher), unter Umständen also auch nach der 24- bzw. 18stündigen Versuchszeit in den Wahlkäfigversuchen. Deshalb entsteht das klarste und schärfste Bild des Präferenz-Nonpräferenzmechanismus bei einem Vergleich der Prozentsätze jener Mutterläuse, die bis zur Reife ihrer erstgeborenen Tochter in der Initialkolonie blieben und Junge produzierten (Tab. II, Zeile 3). Die Unterschiede zwischen R-Reis und R-Vollpflanze einerseits (4,1%) und S-Reis und S-Pflanze andererseits (7,1%) sind dabei geringfügig gegenüber den erheblichen Differenzen zwischen R-Pflanze und S-Pflanze (mit fast 30%) und zwischen R-Reis und S-Reis (mit 26%). Oder anders ausgedrückt: auf dem S-Reis siedeln sich für die Dauer einer Erstgeborenen-Generation nicht viel (7,1%) weniger Mutterläuse an als auf den reinen S-Pflanzen und auf dem R-Reis trotz der S-Unterlage etwas weniger (4,1%) als auf der R-Vollpflanze. Der um ein Vielfaches größere Unterschied zwischen R und S (29.2%) bleibt indessen auch dann bestehen, wenn sie auf einer S- bzw. R-Unterlage wachsen (26,2%). Es zeigt sich also auch hier wieder, daß die Unterlage auf das Präferenzverhalten offensichtlich keinen wesentlichen Einfluß ausübt. Das wird am deutlichsten durch die auf $\frac{R}{S}$ gegenüber R verringerte Ansiedlung bewiesen; die sonst bevorzugte S vermag als Unterlage die Präferenz von R nicht zu verbessern. Die bei R wie bei S gleichsinnige Verminderung der Ansiedlung nach Pfropfung auf die alternative Unterlage läßt sich deshalb kaum anders als ein durch die Pfropfung selbst bedingter Effekt deuten (s.u.).

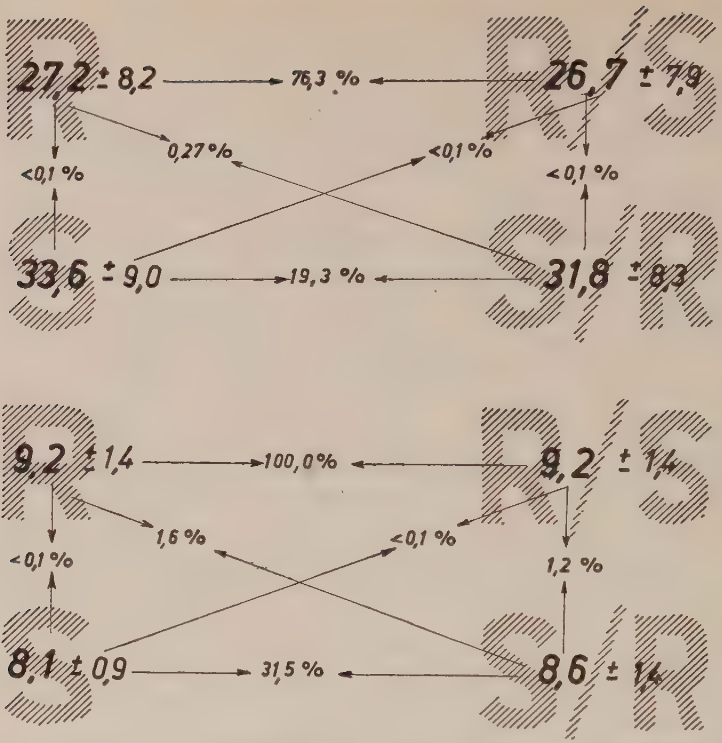

Abb. 4. Antibiosiseffekte auf Pfropfreisern und Kontrollpflanzen (schraffiert). Unten: Durchschnittlich von der Geburt bis zur Reife der Erstgeborenen verstreichende Zeit in Tagen. Oben: Anzahl der in diesem Zeitraum von einer ungeflügelten Virginoparen von *Aphis fabae* auf Normalpflanzen und Pfropfreisern produzierten Töchter. Zwischen den Mittelwerten die Grenzen der statistischen Sicherheit in Prozent.

Ganz ähnliche Verhältnisse ergeben sich bei der Betrachtung der antibiotischen Resistenz, für die Anzahl, Größe und Dauer nur derjenigen Erstgeborenen-Generationen als Maßstab verwendet wurden, deren Mütter die Reife ihrer ältesten Tochter erlebt. Ein besonders deutliches Bild zeigt dabei die durchschnittliche Größe, d.h. die Anzahl der zwischen Geburt und Reife der ersten Tochter geborenen Nachkommen (Abb. 4 oben). Den höchsten Wert weisen die Schlanstedter Pflanzen auf (33,6 ± 9,0). Es folgen mit nur sehr geringem Abstand die auf den $\frac{S}{R}$-Pfropfungen herangewachsenen Familien, die sich statistisch nicht von den S-Familien unterscheiden. Statistisch signifikant viel kleiner sind die entsprechenden Nachkommenschaften auf R und $\frac{R}{S}$, obwohl sie zu ihrer Entwicklung fast einen Tag länger benötigten (s.u.). Untereinander sind sie statistisch nicht verschieden, wenn auch hier, wie in der $\frac{S}{R}$: S-Gruppe, immer die kleineren Werte auf den Pfropfungen entstehen. Es ergibt sich also für die Reproduktionsrate- und geschwindigkeit ein ganz ähnliches Gefälle von S über $\frac{S}{R}$ und R nach $\frac{R}{S}$ wie für die Besiedlungsquote, wobei eine signifikante Zäsur nur zwischen der S- und der R-Gruppe zu erkennen ist. Auch der abiotisch bedingte Resistenzunterschied zwischen R und S bleibt also (nahezu unverändert) erhalten, wenn sich die Kolonien auf Pflanzen entwickeln, die auf einer in ihrem antibiotischen Effekt konträren Unterlage wachsen. Wenn die Unterlage einen Einfluß ausübte, so müßte die Vermehrungsrate auf $\frac{R}{S}$ höher sein als auf R. Da das Umgekehrte der Fall ist, kann es sich nur um einen allgemeinen Pfropfeffekt handeln. Dieser ist beim Übergang von S zu $\frac{S}{R}$ noch größer, was wohl darauf beruhen könnte, daß die auf S optimale Produktion anfälliger ist gegen die durch die Pfropfung erzeugte Verschlechterung der Ernährungsbedingungen und als die ohnehin geringe auf R.

(Verglichen mit früheren Ergebnissen sind die hier vorliegenden Reproduktionsraten auch auf S relativ niedrig. Das könnte dadurch bedingt sein, daß auch die Kontrollpflanzen etwas vergeilt und weniger kräftig waren als in den früheren Versuchen, beruht aber wohl auf einer Minderung der Sortenqualität infolge mehrjährigen Nachbaus.)

Die gleichen Verhältnisse werden bei einem Vergleich der durchschnittlichen Dauer der Erstgeborenen-Gruppen sichtbar (Abb. 4 unten). Die auf R und $\frac{R}{S}$ aufwachsenden benötigen im Mittel signifikant einen halben bis einen Tag länger als die sich auf S bzw. $\frac{S}{R}$ entwickelnden, während sich zwischen R und $\frac{R}{S}$ sowie zwischen S und $\frac{S}{R}$ statistisch keine Unterschiede ergeben.

DISKUSSION

Die vorgetragenen Befunde bestätigen die von HARVEY & HACKEROTT (1958) an Luzernepfropfungen gewonnen Ergebnisse, nach denen sich Überlebens- und Reproduktionsrate von *Therioaphis maculata* weder an der Unterlage noch am Reis gegenüber ungepfropften Normalpflanzen auch nach Monaten veränderten. Allerdings wurden an den Ackerbohnenpfropfungen nur die Reiser geprüft. Bei holzigen Gewächsen ist die Unveränderlichkeit der Resistenzeigenschaften der Pfropfpartner gegenüber Blattläusen seit langem bekannt. Die biologische Bekämpfung von Reb- und Blutläusen macht sich seit Jahrzehnten die Tatsache zunutze, daß wurzelresistente Reben- und Apfelsorten ihre relative Blattlausunanfälligkeit als Unterlagen auch dann unverändert behalten, wenn sie blattanfällige (aber ertragreiche) Sorten tragen.

Bei den bereits vorliegenden Befunden handelt es sich aber nur um Fälle, bei denen die Resistenz ausschließlich auf antibiotischen Wirkungen der Wirtspflanze beruht, oder doch zumindesten bei den Pfropfungen nur diese Komponente geprüft wurde, denn durch Feststellung der Überlebensrate der Aphiden („survival") lassen sich antibiotische und Präferenzeffekte nicht trennen.

Bei der raschwüchsigen, einjährigen Ackerbohne zeigt sich nun nicht nur die gleiche Unbeeinflußbarkeit der antibiotischen Eigenschaften des Reises durch die Unterlage. Auch der Präferenz-Nonpräferenzmechanismus wird durch die Pfropfung nicht verändert. Das prozentuale S : R-Verhältnis ist bei den Initialkoloniegründungen der Käfigwahlversuche (Tab. I, letzte Spalte) in der Wahlkombination $\frac{S}{R}$: R mit ca. 4 : 1 allerdings etwas größer als mit 2,6 : 1 bei der Kombination $\frac{R}{S}$: S. Diese Verschiebung der Häufigkeitsverteilung zugunsten von R im Falle der Pfropfung $\frac{R}{S}$ könnte als ein gewisser Einfluß der S-Unterlage auf die Präferenzeigenschaften des Reises gedeutet werden. Dabei ist jedoch zweierlei zu bedenken. Erstens schwanken die Wahlversuche in Käfigen mehr oder weniger, so daß erst ein noch größeres Material beweisen könnte, ob diese Unterschiede real sind. Bei 24stündigen Kontrollen der Verteilung von Freiland-Initialkolonien schwankte das mittlere R : S-verhaltnis im Verlaufe mehrjähriger Beobachtungsreihen ebenfalls häufig zwischen 1 : 2,5 und 1 : 4,9 (MÜLLER 1951). Zweitens ergab sich bei der Benutzung vollständiger Tochterfamilien als Kriterium (siehe S. 164) keine solche Verschiebung zugunsten der R-Reiser, so daß man annehmen muß, daß durch nachträgliche Abwanderung sich auch in den Käfigen das R-S-Wahlverhältnis noch „normalisiert" hätte, wenn sie länger als 24 Std unter Beobachtung gehalten worden wären.

Nach den Untersuchungen von AUCLAIR, MALTAIS & CARTIER (1957) über die stoffwechselphysiologischen Unterschiede gegen *Acyrthosiphon* anfälliger bzw. resistenter Erbsensorten kann man annehmen, daß die antibiotischen Resistenzunterschiede zumindesten eine quantitative stoffliche Grundlage haben. Die Befunde von Harvey & Hackerott an Luzernepfropfungen sowie die vorliegenden Ergebnisse an der *Aphis fabae*-Resistenz von *Vicia faba*-Pfropfungen zeigen weiter, daß es sich dabei jedenfalls nicht um Stoffe handeln kann, die von der Wurzel geliefert oder in ihrem chemisch-physiologischen Aufbau von ihr beeinflußt werden. Vielmehr muß es sich um Prinzipien handeln, die an den Orten der Nahrungsaufnahme bzw. ihrer Umgebung entstehen. Da *Aphis fabae* zweifellos vorwiegend Phloemsauger ist und sich ebenso gut an Blättern wie an Sproßteilen ernähren kann, dürfte es sich um Substanzen handeln, die in den Blättern gebildet werden.

Die Tatsache, daß sich der antibiotische und der Präferenzmechanismus auf den Pfropfungen gleichsinnig verhalten, berechtigt unseres Erachtens zwar nicht unbedingt zu dem Schluß, daß beide auf dem gleichen Prinzip beruhen, doch erscheint es nicht ausgeschlossen, daß sie letztlich ursächlich zusammenhängen. Die Untersuchungen der letzten Jahre (JOHNSON 1958, MITTLER 1957, KENNEDY 1958, 1959, MÜLLER 1958, HENNIG 1959) deuten immer mehr darauf hin, daß für die Wirtswahl der Aphiden, mindestens der polyphagen *Aphis fabae* und *Myzodes persicae* Sulz., weder die sogenannten Probesaugstiche noch vorübergehende Ansiedlung die entscheidende Rolle spielen. Zwar können dabei schon einzelne Junglarven abgesetzt werden, aber eine endgültige Ansiedlung kommt offenbar erst zustande, wenn die Blattlaus einen Platz gefunden hat, an dem sie leicht hochwertigen Nahrungssaft gewinnen kann. Die Chance hierfür erscheint rein statistisch auf den resistenten Sorten geringer zu sein als auf anfälligen, indem raschwüchsige, nährstoffreiche Organe auf ihnen verschieden verteilt und verschieden häufig sind (TAMBS-LYCHE & KENNEDY 1958). Die Läuse haben jedoch offenbar keine Möglichkeit, günstige Stellen äußerlich zu erkennen und können eine Entscheidung erst nach längerem Saugen treffen. Damit würden sich die Unterschiede zwischen Wirtswahl bzw. Ansiedlung und Reproduktionsrate weitgehend verwischen und auf ein Prinzip — das der Nahrungsqualität — zurückführen lassen. Es wird damit auch verständlich, daß Wirtswahl- und Vermehrungsunterschiede um so deutlicher werden, je mehr und je länger die Läuse (an isolierten, auf ein Blatt zurückgeschnittenen Pflanzen oder in Käfigversuchen) gezwungen werden, sich auf identischen Organen der verglichenen Sorten anzusiedeln und auf ihnen Junge abzusetzen. Diese Vorstellung ließe es dann auch ganz verständlich erscheinen, daß sich Präferenz- und Antibiosiserscheinungen auf Pfropfungen gleichsinnig verhalten.

Die gleichsinnige Minderung der Ansiedlungsquote sowie der Reproduktionsrate und -geschwindigkeit auf $\frac{R}{S}$- wie auf $\frac{S}{R}$-Pfropfungen (im Gegensatz zu den Normalpflanzen) kann dann als Verschlechterung der Ernährungsbedingungen und damit der Wirtswahl- und Reproduktionsrate gedeutet werden, die auf der erschwerten Stoffleitung (Druckunterschiede) in den Gefäßen zwischen den Pfropfpartnern beruhen könnte. Selbst wenn man annimmt, daß doch andere Faktoren: Textur, Oberflächenbeschaffenheit, sekundäre Reizstoffe (Repellents, Attrakivstoffe) über die erste Ansiedlung entscheiden, so könnten die Druckverhältnisse in den Pflanzen zumindesten einen Einfluß auf die sekundäre Abwanderung (d.h. nach vorübergehendem Saugen oder selbst nach Absetzen von ein oder wenigen Junglarven) und damit auf die endgültige Befallsrate ausüben.

SUMMARY

APHID RESISTANCE OF SCIONS FROM RECIPROCALLY GRAFTED RESISTANT AND SUSCEPTIBLE FIELD BEANS

Reciprocal grafts of aphid-resistant (Rastatter) and -susceptible (Schlanstedter) field beans (*Vicia faba*) should demonstrate whether the resistance of the scions is influenced by the stock or not.

To ensure a solid and inflexible connection between the partners to be grafted a glass capillary was fitted in the stem hollows of both the scion and the stock.

In "pertelar" cages (MÜLLER 1954) one young aptera of *Aphis fabae* could choose to infest a leaf of a grafted scion or a leaf of a normal plant. The percentage of initial colonies established by 740 such tested virginoparae decreased from 90,6 ($\frac{S}{R}$ + S) to 78,5 ($\frac{S}{R}$ +R), 76,7 ($\frac{R}{S}$ + S) and 48,8% ($\frac{R}{S}$ + R). In all cases, where a decision was possible at all, S-leafs were preferred to R-leafs, independently of whether they grew on a scion or on a normal plant (Table I). With reproduction rate experiments the percent of apterous virginoparae settling on grafts and controls dropped in a similar manner from 51,0 on S and 43,9 on $\frac{S}{R}$ to 21,8 on R and 17,7 on $\frac{R}{S}$ respectively (Table II).

Also the reproductive rate (number of larvae born by an apterous virginopara in the time between the birth and maturity of its first born daughter decreased from S (33,6 ± 9,0) and $\frac{S}{R}$ (31,8 ± 8,3) to R (27,2 ± 8,2) and $\frac{R}{S}$ (26,7 ± 7,9). There is no statistically significant difference between S and $\frac{S}{R}$ on the one side and R and $\frac{R}{S}$ on the other (Fig. 4). The diminution of numbers from S to $\frac{S}{R}$ and from R to $\frac{R}{S}$ respectively in all experiments is suggested to be an effect of grafting in itself rather than a mutual influence of the partners.

The present results prove that both the preference-nonpreference- and the antibiosis-mechanism of the relative aphid resistance of R- and S-field beans are not altered by the

stock after reciprocal grafting. In view of this and the findings of HARVEY & HACKEROTT (1958) on alfalfa resistance against *Therioaphis maculata* substances produced by the roots cannot be the cause of aphid resistance.

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BOOK REVIEWS

CHRISTOPHERS, S. R. *Aedes aegypti. The Yellow Fever Mosquito: its life history, bionomics and structure.* Cambridge University Press, London 1960, pp. XII + 739, 86 figs.

This large book of 739 Crown Quarto pages, over 300,000 words, weighing almost 2 kilograms and costing £ 3-15-0 (even though its production is subsidised by the Rockefeller Foundation) may, to some, seem an unnecessarily detailed study of a single insect species. I do not think this criticism is well founded. Quite apart from the importance of the yellow fever mosquito in public health, this insect is used in laboratories throughout the world for work on insect physiology and insecticides. Everyone who works with *Aedes aegypti* in the field or in the laboratory will learn much from this book, and none will wish it were one page shorter than it is. It is clearly and succinctly written, easy to follow but with no unnecessary padding. It is primarily a reference book for the specialist, but is recommended to the attention of a wider audience as an admirable illustration of many fundamental principles in ecology, physiology and systematics.

The book starts with an historical introduction, and then deals with systematics and synonomy. I can remember the days when, in lectures on medical entomology, we referred to "the yellow fever mosquito" or sometimes "the 'stegomyia' mosquito", and hesitated to use its scientific name in case that had been changed overnight. It is comforting to find that it is now agreed to retain the name *Aedes aegypti.* The account of varieties and strains, of their distribution and habitats, does much to clear up the confusion which still confuses the literature. There is a short chapter on the importance of *Aedes aegypti* as a carrier of the virus of yellow fever, and this includes a useful summary of rural and sylvan yellow fever and of the other mosquitoes concerned. The implication of this insect in carrying dengue and other diseases is also discussed. Some readers may find this medical section too short, though they will be hard put to point out any serious omissions. As Sir Rickard Christophers is himself a distinguished medical man as well as an entomologist, his emphasis on the entomological rather than on the medical side of his subject is deliberate, and justified by the results; it is clearly not due to any lack of knowledge of tropical medicine!

The major part of the book deals with the internal and external anatomy of all stages in the life history, and with the physiology of all these stages. It is impossible to summarise this which is already a summary of work described in many hundreds of papers listed in the references. There are 86 clear line drawings and 50 tables to illustrate the text. No photographs are included; the reader is not conscious of any need for any, nor for any further illustrations.

Sir Rickard Christophers undertook a formidable task in bringing together the vast amount of information scattered throughout the literature; I know of no other writer who could have accomplished this half so well. There are omissions of course. Like most reviewers I have searched for references to my own work, and can find no account of several papers which I might have expected to see included, but nevertheless my admiration for his achievement in covering the essentials of his subject is in no way diminished.

KENNETH MELLANBY

ARTHUR, D. R. *Ticks Part V. The genera Dermacentor, Anocentor, Cosmiomma, Boophilus, Margaropus.* Cambridge University Press, London 1960. pp. xviii + 251, 4 Plates, 510 figs., £ 3.

The first four parts of the monograph on the ticks planned by the late Professor G. H. F. Nuttall and written by him and his colleagues C. Warburton, W. F. Cooper and L. E. Robinson, and dealing with the Argasidae and the Ixodidae, appeared between 1908 and 1926. Now in 1960 Dr. Don R. Arthur has produced volume V, dealing mainly with the genus *Dermacentor.* Each species considered is described in detail, the literature is reviewed, and what is known of the biology and importance in disease transmission is summarised. The illustrations are excellent. This book is well worthy to stand alongside the earlier volumes.

KENNETH MELLANBY

Frank Wilson : *A review of the biological control of insects and weeds in Australia and Australian New Guinea.* Technical Communication No. 1, Commonwealth Institute of Biological Control, Ottawa, Canada. Published by the Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England. 104 pp., 1960, 25s.

The Continent, Archipelagoes and Islands in the Pacific Area are very well situated for experimental work on Biological Control of Insects and Weeds. However, most of the work is published in local papers, reports or communications, or it is not published at all. Therefore it is very gratifying that the Commonwealth Institute of Biological Control has started a Series of publications which will contain reviews of work in different parts of the Commonwealth. Three quarters of this book are devoted to insect problems. The investigations dealt with are very different in value: some protracted and thorough, very often purely empirical, and some brief and superficial. The author nevertheless gives a list of introduced parasites of insects divided into five categories: (1) Pests substantially reduced in status; 8 cases; (2) Pests reduced in status, 11 cases; (3) Pests of doubtfully diminished status, 4 cases; (4) Pests of unchanged status, 4 cases; (5) Introduction of parasites not leading to establishment, 12 cases.

One quarter of the book deals with the control of 10 species of weeds, of which the results against Prickly pear (*Opuntia* spp.), St. John's wort (*Hypericum perforatum* L. var *angustifolium* DC), and Lantana (*L. camara* L.) are well known.

A special chapter discusses the question whether exotic or native pests are the best subjects for biological control. Useful results have been obtained with twelve out of twenty-six in the exotic category, three out of four in the cosmopolitan category, and only one out of six in the native category. The weed control measures all concern exotic weeds.

This very critical review is also valuable because of the extensive bibliography included.

P. A. van der Laan

Ent. exp. & appl. 3 (1960): 173—184. *North-Holland Publishing Co., Amsterdam*

DAMAGE TO THE OAT PANICLE BY THE FRIT FLY

BY

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About 2,000 panicles (60,000 spikelets) of known bursting date from small plots of oats were dissected in detail. It is noted that spraying young oat plants with DDT against first generation attack also reduces panicle attack. The spraying causes physical differences between the sprayed and the unsprayed areas — namely spraying gives a higher plant density and uniformity of panicle bursting date. The liability of main grain rather than a bosom grain to be attacked is noted at different levels of overall attack.

Blindness of oats is discussed, and data are given to support the theory that one type of blindness is due to early frit fly attack on the immature grain.

In southern England, the frit fly (*Oscinella frit* L.) has three generations a year, the second generation attacking the young grain of oats. CUNLIFFE (1921—1936) did much basic work on the frit fly, noting the fluctuations in adult numbers and the damage done by the larva. FRYER & COLLIN (1924) noted when the oat grain was most susceptible to attack. CUNLIFFE & FRYER (1924) and SHEALS (1950) considered the frit fly to be of little or no importance in causing blindness of oats. Recent work on the panicle attack includes that of EMPSON (1957), JEPSON & SOUTHWOOD (1958) and STRICKLAND (1959). Notwithstanding the advances in knowledge described in these papers, much has yet to be learned about this stage in the life history of the frit fly, and the work described here was planned to try to fill some of the gaps.

Small plots of oats were sown on Rothamsted farm in 1958. Six varieties were sown, two adjacent rows of each variety, 18 cm apart; the rows being about 4 m long. Four such plots were sown, the first sowing being on April 10th, the successive sowings being at six day intervals. The varieties sown were Eagle, Sun II, Von Lochows Yellow, Star, Summer and Victory. Ibbotson sticky traps (IBBOTSON 1958) painted with white Valspar enamel were placed one in each plot at crop height and changed twice weekly. Half of each plot was sprayed twice with D.D.T. against the first generation attack, the second spray being applied a fortnight after the first. Panicles were bursting between mid June and mid July over the four plots. During this period, panicles were marked by slipping a small label around the stem when the spikelets were just beginning to burst out of the leaf sheath (see Fig. 1). The date and the number of spikelets showing were noted.

Marking was done between June 25th and July 20th. The early bursting panicles on plot I were not marked, nor was the variety Summer which was well advanced

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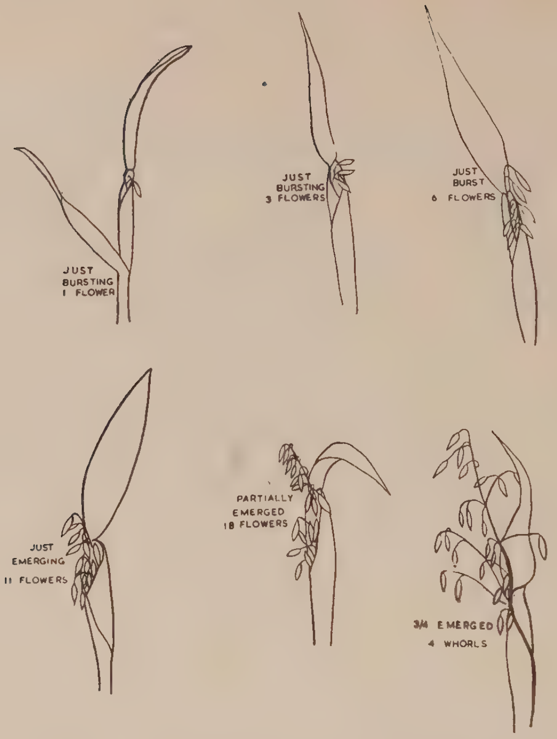

Fig. 1. The development of a typical panicle, from "just bursting" to "¾ emerged", takes from four to seven days according to the size of the panicle and the temperature. The majority of panicles burst from the apex downwards. A few burst sideways, these usually being exceptionally large panicles.

on plots I and II, and on plot III 50% of the panicles of Summer had burst by June 25th. Earlier panicles could not be marked owing to the pressure of other work. As many panicles as possible were marked each day, all the plots being covered. The majority of panicles marked fell into the date range July 2nd to July 8th.

Care was taken to move as carefully as possible among the plants. It was impossible to avoid disturbing the more advanced panicles while labelling later ones. All plots and varieties were presumed to be disturbed equally. The disturbance caused was considered to be no more than that occurring in a moderate wind.

Unfortunately, due to the wet weather of July and August, many of the labels written with a ball point pen or pencil became illegible by harvest time. After harvesting, the sheaves of oats were carried into an insectary and the labelled panicles were put into individual bags and stored prior to dissection.

During the winter of 1958—59, over two thousand labelled panicles were fully dissected and the details of each grain were recorded, as the example on Table I. The kernel of each grain was removed from the husk and its condition noted. The grains were classified as indicated in the key under the Table.

Husks containing a minute, unfertilized ovule and husks containing nothing and showing no sign of insect damage were classified as blind.

TABLE I

Example of the dissection details recorded for a panicle
Eagle Plot I Sprayed
29/6 just bursting

	Whorl 5	Whorl 4	Whorl 3	Whorl 2	Whorl 1
sb1.	sp1 mg H bg H sp2 mg H bg H sp3 mg PA bg H sp4 mg H bg H	sp1 mg H bg H tg H sp2 mg H bg H	sp1 mg H bg H tg B sp2 mg H bg H tg H	sp1 mg H bg H tg B	sp1 mg H bg H tg H
sb2.	sp1 mg H bg H sp2 mg H bg B sp3 mg H bg H sp4 mg H bg H	sp1 mg H bg H sp2 mg H bg H	sp1 mg H bg H	sp1 mg H bg H tg H	sp1 mg H bg PA
sb3.	sp1 mg H bg H sp2 mg H bg H	sp1 mg H bg H	sp1 mg H bg H tg H	—	sp1 mg PA bg H
sb4.	sp1 mg H bg H sp2 mg H bg H	sp1 mg H bg H	—	—	—
sb5.	sp1 mg H bg H	sp1 mg H bg H	—	—	—
sb6.	sp1 mg H bg H	sp1 mg H bg H	—	—	—
sb7.	sp1 mg H bg H	sp1 mg H bg H	—	—	—
sb8.	sp1 mg PA bg H	sp1 DG mg B bg H			

Whorl 1 = apical whorl.
Whorl 5 = basal whorl in this example.
sb = side branches. Order of numbering has no significance.
sp = spikelet. Numbers go from base to apex of side branch.
mg = main grain.
bg = bosom grain.
tg = tertiary grain.
H = healthy grain (this also includes a very small number of mechanically damaged and shrivelled grains).
A = attacked grain (i.e. whole grain fritted).
PA = partially attacked (i.e. only part of grain fritted).
B = blind grain.
DG = double grain where the bosom grain is inside the husk of the main grain.

TABLE II

Data for Panicles Bursting 25/6—1/7

Sowing Date	No. of Pani-cles	Av. No. Grains/ Panicle	Total No. of Grains	Grains "H"	Grains "A"	% Grains "A"	Grains "B"	% Grains "B"	Av. Bursting Date	No. of Panicles	Av. No. Grains/ Panicles	Total No. of Grains	Grains "H"	Grains "A"	% Grains "A"	Grains "B"	% Grains "B"	Av. Bursting Date
Sun II sprayed										*Sun II unsprayed*								
10/4	24	59.5	1427	1288	62	4.6	77	5.4	28.5	6	96.3	578	473	57	10.8	48	8.3	29.8
16/4	4	60.25	241	215	7	3.2	19	7.9	29.5	7	88.0	616	542	37	6.4	37	6.0	30.0
Von Lochows Yellow sprayed										*Von Lochows Yellow unsprayed*								
10/4	13	66.9	868	792	49	5.8	27	3.1	28.9	4	92.3	369	326	31	8.7	12	6.7	29.8
16/4	6	59.5	357	315	18	5.4	24	6.7	29.3	3	127.3	382	361	12	3.2	9	2.4	30.3
Star sprayed										*Star unsprayed*								
10/4	8	59.4	475	393	30	7.1	52	10.9	29.8	8	80.4	643	525	37	6.6	81	12.6	29.9
16/4	6	60.7	364	311	21	6.3	32	8.8	29.2	14	76.4	1070	887	60	6.3	123	11.5	28.8
Victory sprayed										*Victory unsprayed*								
10/4	4	60.3	241	200	16	7.4	25	10.4	30.0	1	51.0	51	36	5	12.2	10	19.6	26.0
16/4	10	85.9	859	791	35	4.2	33	3.8	30.2	6	116.3	698	594	76	11.3	28	4.7	29.2
Summer sprayed										*Summer unsprayed*								
28/4	33	48.0	1584	1408	115	7.6	61	3.9	29.5	29	54.3	1574	1272	191	13.1	111	7.1	29.2

TABLE III

Data for Panicles Bursting 2/7—8/7

Sowing Date	No. of Panicles	Av. No. Grains/ Panicles	Total No. of Grains	Grains "H"	Grains "A"	% Grains "A"	Grains "B"	% Grains "B"	Av. Bursting Date	No. of Pani- cles	Av. No. Grains/ Panicle	Total No. of Grains	Grains "H"	Grains "A"	% Grains "A"	Grains "B"	% Grains "B"	Av. Bursting Date
Eagle sprayed										*Eagle unsprayed*								
10/4	28	63.5	1777	1488	188	11.2	101	5.7	4.0	8	49.3	394	300	64	17.6	30	7.6	4.2
16/4	47	74.6	3508	2375	366	11.0	167	4.8	4.8	16	78.1	1250	931	230	19.8	89	7.1	4.1
22/4	75	70.8	5312	4153	894	17.7	265	5.0	6.4	10	74.9	749	545	163	23.0	41	5.5	6.8
28/4	19	64.7	1229	913	270	22.8	46	3.7	7.4	3	66.0	198	142	38	21.1	18	9.1	7.3
Sun II sprayed										*Sun II unsprayed*								
10/4	16	54.3	868	737	56	7.1	75	8.6	3.7	16	57.6	921	619	175	22.0	127	13.8	3.6
16/4	30	71.7	2151	1853	138	6.9	160	7.4	4.7	24	73.8	1772	1438	213	12.9	121	6.8	4.3
22/4	127	70.9	9001	7521	933	11.0	547	6.1	5.5	25	79.6	1990	1515	271	15.2	204	10.3	5.4
28/4	25	67.1	1677	1361	205	13.1	111	6.6	5.6	16	65.1	1042	784	184	19.0	75	7.1	6.3
Von Lochows Yellow sprayed										*Von Lochows Yellow unsprayed*								
10/4	2	34.0	68	59	8	11.9	1	1.5	5.0	12	74.8	898	744	87	10.5	67	7.5	4.5
16/4	20	56.8	1136	994	101	9.2	41	3.6	3.7	22	79.3	1745	1493	188	11.2	64	3.7	3.6
22/4	125	70.8	8851	7697	874	10.2	280	3.2	6.4	29	73.7	2136	1592	368	18.8	176	8.2	5.9
28/4	31	64.1	1986	1605	292	15.4	89	4.5	6.7	10	80.4	804	587	160	21.4	57	7.1	7.1
Star sprayed										*Star unsprayed*								
10/4	8	30.1	241	185	10	5.1	46	19.1	3.4	10	65.5	655	478	76	13.7	101	15.4	4.6
16/4	12	55.3	663	570	41	6.7	52	7.8	3.7	17	83.6	1421	1117	137	10.9	167	11.8	4.2
22/4	147	62.3	9163	7693	805	9.5	665	7.3	4.2	20	82.95	1659	1312	181	12.1	166	10.0	4.2
28/4	12	92.8	1114	857	128	13.0	129	11.6	7.4	8	76.5	612	398	105	20.9	109	17.8	5.9
Summer sprayed										*Summer unsprayed*								
10/4	1	46.0	46	38	2	5.0	6	13.0	3.0	4	54.0	216	165	22	11.8	29	13.4	4.0
16/4	0	—	—	—	—	—	—	—	—	8	43.0	344	266	60	18.4	29	5.2	3.4
22/4	43	36.1	1553	1412	61	4.1	80	5.2	2.9	43	34.9	1502	1101	180	14.1	221	14.9	3.3
28/4	42	35.6	1495	1266	151	10.7	78	5.2	5.0	25	42.6	1066	721	269	27.2	76	7.1	4.5
Victory sprayed										*Victory unsprayed*								
10/4	36	60.1	2162	1885	133	6.6	144	6.7	4.0	2	29.0	58	30	7	18.9	21	36.2	5.0
16/4	54	68.6	3702	3194	310	8.8	198	5.3	4.1	16	65.9	1054	849	123	12.7	82	7.8	4.9
22/4	42	68.2	2865	2217	382	14.7	266	9.3	5.2	4	99.25	397	298	75	20.1	24	6.0	5.0
28/4	21	75.6	1588	1141	337	22.8	110	6.9	7.2	2	101.5	203	118	65	35.5	20	9.9	7.0

TABLE IV

Data for Panicles Bursting 9/7—18/7

Sowing Date	No. of Pani-cles	Av. No. Grains Panicle	Total No. of Grains	Grains "H"	Grains "A"	% Grains "A"	Grains "B"	% Grains "B"	Av. Bursting Date	No. of Panicles	Av. No. Grains Panicle	Total No. of Grains	Grains "H"	Grains "A"	% Grains "A"	Grains "B"	% Grains "B"	Av. Bursting Date
					Eagle sprayed									*Eagle unsprayed*				
22/4	37	71.1	2629	1990	486	19.6	153	5.8	10.8	36	51.7	1862	1214	419	25.7	229	12.3	13.6
								Data for Panicles Bursting 13/7—19/7										
					Eagle sprayed									*Eagle unsprayed*				
28/4	22	48.2	1061	752	227	23.2	82	7.7	15.5	8	43.9	351	179	105	37.0	67	19.1	17.1
					Sun II sprayed									*Sun II unsprayed*				
28/4	3	24.0	72	46	8	14.8	18	25.0	15.3	13	38.8	505	284	102	26.4	119	23.6	16.8
					Von Lochows Yellow sprayed									*Von Lochows Yellow unsprayed*				
28/4	14	53.1	743	548	122	17.3	37	5.0	14.4	25	55.1	1378	798	438	35.4	142	10.3	16.8
					Star sprayed									*Star unsprayed*				
28/4	3	36.0	108	68	18	23.7	22	20.4	15.3	28	49.3	1381	713	368	34.0	300	21.7	16.5
					Summer sprayed									*Summer unsprayed*				
28/4	0	—	—	—	—	—	—	—	—	1	14.0	14	10	3	23.1	1	7.1	15.0
					Victory sprayed									*Victory unsprayed*				
28/4	9	43.6	402	196	103	34.4	103	25.6	15.8	36	54.8	1962	852	667	43.9	443	22.6	16.1

The whorls of the panicle were numbered from the apex (whorl I) to the base.

Nearly all the results given here are each based on at least one thousand grains.

Tables II to IV show the results obtained from the panicle dissection. Several points may be noticed, first, the percentage attack on the unsprayed plots on panicles bursting at the same time is consistently higher than the percentage attack on the sprayed plots. This difference cannot be due to any D.D.T. residues as the last spray was six weeks earlier than panicle burst. The only other obvious difference between the sprayed and the unsprayed areas was the panicle density which was much higher where the plants were sprayed. This means that there were more susceptible panicles per square yard in the sprayed areas, and the adult flies, which may be considered to be moving randomly throughout such a small area of oats, are less likely to land on any one grain in a susceptible condition in the more dense areas.

Secondly, the percentage attack increases when the sowing date is sometimes later. This is also considered to be an effect of panicle density and the physical conditions by which the panicle is surrounded.

Thirdly, the percentage blindness is also higher on the unsprayed plots than on the sprayed ones, but does not show an increase on the later sown plots.

If blindness is partially due to an early frit attack on grains not far enough developed to support a larva, a higher percentage blindness may be expected where there is a higher percentage attack. It is possible that the tiller generation attack may weaken the plant, so making less food supplies available for the panicle. If this were true, it may be expected that the weakened plants would give rise to smaller panicles, whereas the reverse is true.

Fourthly, panicles on the unsprayed plots, especially the later sown ones, are larger than on the sprayed plots. Panicles bursting on the unsprayed plots during this period were developed from primary tillers, having escaped tiller generation attack. These relatively few primary tillers seem to benefit from the "thinning" treatment given by the frit-fly, and develop into larger panicles. Panicles which developed from the secondary tillers burst later and were much smaller than the early bursting ones (Fig. 1). The variety Summer has much smaller panicles than any other variety used in these experiment.

Fifthly, there is a varietal difference in the percentage of blind grains occurring, Star appears more liable and Eagle and Von Lochows Yellow least liable to blindness c.f. CUNCLIFFE (1930), EMPSON (1957).

THE RELATIONSHIP BETWEEN THE ATTACK ON THE MAIN GRAIN AND ON THE BOSOM GRAIN

EMPSON (1957) and STRICKLAND (1959) both state that the ratio of the percentage of main grains attacked to the percentage of bosom grains attacked diminishes as the overall attack increases. They give a ratio of 4 : 1 for an overall attack below ten per cent and approaching 1 : 1 above a sixty per cent overall attack.

The results of this experiment verified these figures, ratios of between 5.6 : 1 for a 6 per cent overall attack and 1.8 : 1 for a 57 per cent overall attack were obtained.

The results obtained from approximately eight hundred panicles were examined more closely. Spikelets were divided into eight categories, according to the condition of the main and the bosom grains; and the numbers of each type of spikelet occurring in every whorl of the panicles were noted.

It was apparent that there were more main grains attacked than bosom grains, and that attacked bosom grains associated with attacked main grains occur far more frequently than would occur if distribution of attacked bosom grains was at random. This association was found to be highly significant on all plots, sprayed or unsprayed, and with all varieties examined (Table V shows the results for Von Lochows Yellow; the X^2 test indicated that the probability that the attacked bosom grains are associated with attacked main grains exceeds 0.001).

TABLE V

Von Lochows Yellow Plot III Sprayed Panicles Burst July 7th. 48 Panicles

Whorl	Total Spikelets	$\frac{H}{H}$	$\frac{A}{H}$	$\frac{H}{A}$	$\frac{A}{A}$	$\frac{B}{H}$	$\frac{H}{B}$	$\frac{B}{B}$	$\frac{A}{B}$
1	107	89	10	3	2	1	1	1	0
2	108	95	8	2	1	1	1	0	0
3	232	195	19	12	2	2	2	0	0
4	420	340	41	28	5	2	3	0	1
5	546	428	64	27	14	3	8	0	2
6	207	162	27	8	4	1	3	0	2
Totals	1620	1309	169	80	28	10	18	1	5

Previous papers on frit fly reveal no attempt to explain this phenomenon.

RIGGERT (1935) states that the frit fly lays its eggs on the glumes and the rachis. The glumes open out when the flower is ready for fertilization, so that if eggs are laid on the glumes or on the rachis the newly hatched larva in its search for a suitable food supply will most likely reach the main grains as this is attached directly to the rachilla or spikelet axis with a truncate or stout base. The bosom grain is tapering and is inserted on the main grain near the base on the grooved side, on its own, very thin, stalk. If a tertiary grain is present, it is inserted in the same manner on the bosom grain. If a newly hatched larva finds the main grain already attacked near the base of the groove, it may go on to seek fresh food material and eventually reach the bosom grain. If a newly hatched larva is on the inner side of either glume, which may be touching the nearest grain, both main and bosom grains would be equally likely to be attacked. The main grain is also bigger than the bosom grain (EMPSON 1957), although the bosom grain is large enough to support a larva until maturity.

BLINDNESS IN OATS

During dissection, all grains which were underdeveloped or which had a normal husk but no kernel were recorded as blind. Underdeveloped grains, which contained a minute ovule, were pale green in colour and had a small narrow husk. Other grains looked normal, but on dissection revealed no sign of a kernel or of insect

TABLE VI

1958 Results of Victory Oats showing relationship between Frit Attack and Blindness

Date of Bursting	Spikelets	$\frac{H}{H}$	$\frac{A}{H}$	$\frac{H}{A}$	$\frac{A}{A}$	$\frac{B}{H}$	$\frac{H}{B}$	$\frac{B}{B}$	$\frac{A}{B}$	$\frac{B}{A}$	A	%A	B	%B	$\frac{B}{A+B}$ Main	$\frac{B}{A+B}$ Bosom
30/6—3/7	1078	892	91	14	6	34	35	3	3	0	120	5.6	75	3.5	.27	.66
3/7—6/7	966	759	90	19	7	38	44	6	0	3	126	6.5	97	5.0	.33	.63
4/7—6/7	2957	2116	409	90	35	140	118	20	20	9	598	10.1	327	5.5	.27	.54
1/7—5/7	431	277	77	15	5	33	17	0	6	2	261	13.0	143	7.1	.26	.55
4/7—5/7	1002	635	177	40	14	61	52	7	12	4	109	12.6	58	6.7	.28	.52
13/7—19/7	1027	211	314	41	121	77	69	57	112	25	734	35.7	397	19.3	.23	.56

damage, and were also recorded as blind. Single grained spikelets recorded as blind very often came into the former category described. Blind grains recorded in a double grainted spikelet more often fell into the second category. It seems very likely that this type of blindness is caused by a very early frit attack, i.e. the larva has attacked the underdeveloped grain, or just after the time of fertilization, and the grain has been unable to continue growth and support the larva.

Cunliffe (1924) reared larvae on grains in small cages and found that they favoured the groove between the pericarp and the endosperm. They sometimes extended feeding to the embryo and the conducting vessels near the base of the seed. When this happened, the seed was dwarfed and the entire endosperm was eaten by the larvae. Larvae could also reach maturity by attacking the ovule immediately after fertilization, when they destroyed all parts of the flower, which was consequently sterile. Cunliffe & Fryer (1924) state that 'very immature seeds, as a result of infestation, may cease growth immediately after fertilization, in which case, the small fly larvae may die or migrate leaving very little evidence of its work'. They classified these grains with the definitely blind ones. In 1930, Cunliffe stated that 'blindness of grain is possibly due to the operation of several factors — weather at the time of fertilization, insect action, variety of oat'. He concluded that the frit fly was not responsible as the percentage grain attacked and the percentage grain blind showed no correlation. With reference to the Table VI the percentages of grains attacked and grains blind increased steadily together. The ratio of grains blind to total grains 'damaged' is approximately constant for main grains and for bosom grains, although the two ratios are distinct. The bosom grain is smaller than the main grain, therefore is more likely to be entirely consumed by a larva (Empson 1957) and it matures at a later date than its corresponding main grain (Fryer & Collin 1924). These facts probably account for the higher ratio of blind grains to total damaged grains among the bosom grains.

With reference to Table VII the percentages of attacked and blind grains also show a close agreement when the results for Victory are examined whorl by whorl.

TABLE VII

Results of Victory Oats bursting from 30/6—19/7 1958

		Percentages Grain Damaged				
		Main		Bosom		
Whorl	No. of Spikelets Examined	Frit Attacked	Blind	Frit Attacked	Blind	Total
1	489	20.4	8.4	5.5	7.8	21.1
2	495	21.1	8.5	7.5	9.1	23.2
3	960	20.7	6.6	7.8	10.1	22.6
4	1825	19.8	7.0	6.2	7.2	20.1
5	2686	19.2	6.0	5.4	6.1	18.3
6	1105	16.5	5.1	2.8	5.3	14.8

In the individual panicles, damage, i.e. attack plus non-physiological blindness, is found anywhere within the panicle. In the bulked results, it becomes clear that the centre of the panicle is slightly more damaged than the apex or the base, which is the least damaged. The basal whorl may be less damaged because it gains some measure of protection from the rest of the panicle above it, i.e. the flies may alight on the base of the panicle less frequently. The difference between the apical whorl and the one immediately below it (whorl 2) may be due to the exposure and extra mobility of the topmost whorl, making it less convenient for the fly to alight there.

ROEBUCK (1920) found larvae feeding in the developing ensheathed panicles, causing the whole panicle to be deformed and mostly blind. SHEALS (1950) also noted this phenomenon and both workers concluded it was due to an intermediate generation of flies. CUNLIFFE & FRYER (1924) did not record an attack of this nature, stating that this 'would appear to be entirely inconsistent with the results obtained in the present experiment'. In 1958, no damage of this nature occurred in the oats grown at Rothamsted. In 1959 however, during very late tiller dissections, this type of damage was occasionally noted, the resulting panicles were completely deformed. The numbers of flies caught during the two years explains this. In 1958, the two to four peaks occurring in each generation, probably caused by cold spells during the first adult generation, produced 'waves' of flies throughout the season. It is likely that this type of damage is caused by flies occurring between the two normal peaks for the tiller and the panicle generations.

Similar experiments done in 1959 yielded confirmation of these results.

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ZUSAMMENFASSUNG

SCHÄDEN AN DER HAFERRISPE DURCH DIE FRITFLIEGE

Ungefähr 2000 Rispen (60000 Ährchen) von kleinen Haferparzellen bekannter Blühdaten wurden genau seziert. Es wurde festgestellt, daß DDT-Spritzungen junger Haferpflanzen gegen die erste Generation der Fritfliege auch den Rispenbefall vermindert. Die Spritzung verursacht physikalische Unterschiede zwischen gespritzten und unbehandelten Flächen—Spritzen ergibt nämlich eine höhere Bestandsdichte und Gleichförmigkeit der Daten des Ährenschiebens. Die größere Befallsgefährdung der Hauptkörner gegenüber den Innenkörner wird auf erschiedenen Stufen des Gesamtbefalls festgestellt.

Die Taubheit des Hafers wird diskutiert und es werden Befunde mitgeteilt, welche die Theorie stützen, daß eine Art der Taubheit auf frühem Fritfliegenbefall der unreifen Körner beruht.

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BIOLOGY OF *TIPULA OLERACEA* L.: GROWTH OF THE LARVA

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The growth of larvae, isolated and in groups, in the laboratory at constant temperature is described. The length of the larval stage varies widely even under uniform conditions. The relationship between larval weight and time is exponential in the first three instars, linear in the fourth. The paper is intended as an introduction to further publications on the causes of variation in growth.

In nature *Tipula oleracea* usually has two generations a year. This much is clear from previous work. DEL GUERCIO (1914) in Italy, observed adults flying in mid-March and again in October-September. MILES (1921) states that in the Midlands and west and south-west England there are two fairly well-marked broods, the first from the end of April to May-June, the second in September-October. In European Russia (Pskov) SILANT'EV (1931) saw adults flying from July to September or October, while in Germany the first flies appeared in May-June, the last in September-October (SELLKE, 1936). In the North of England (MILNE, unpublished data) flies have been trapped between May and October with spring and autumn peaks in numbers caught per day.

Variation in the flight period dates from different regions is probably due to different temperature conditions. Culture of *Tipula oleracea* at various constant temperatures shows that there is no diapause at any stage. Any synchronisation of the generations with the seasons is likely to depend chiefly on seasonal temperature changes.

In the laboratory, at 21° C the complete life cycle takes an average of 11—12 weeks (Egg, 6 days. Larva, 8.8 weeks. Pupa, 1 week. Adult, 3—4 days before mating and oviposition). There is little variation in the egg, pupal and adult stages but the larval stage can vary between 5 and 17 weeks at the one temperature. This paper describes the growth of the larva and the wide variation possible in the length of the larval stage. Future papers will deal with the effect of different physical factors on growth and with the causes of variation.

METHOD

The larvae were kept in sand at 21° C and fed on powdered dried grass as described previously (LAUGHLIN, 1958). Groups of larvae were kept in polythene boxes, 12 cm square and 5 cm deep with a fine meshed phosphor bronze gauze bottom. Individuals were kept in glass tubes with metal gauze caps at both ends or in polystyrene 'honeycomb' boxes in which the larvae were isolated by partitions. For the first three instars the sand was kept wet by standing the containers in 1 cm of water. Fourth instar larvae were kept in damp sand: the boxes or tubes of dry sand were flooded and then allowed to drain for several minutes, standing on blotting paper or a towel.

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Torsion balances were used to weigh the larvae and pupae. The individuals were washed in clean water to remove sand grains and particles of powdered grass, then gently rolled on filter paper to dry the surface. The whole process was very quick and the larvae were out of their tubes for a minute or two only. Neither did they appear to be unduly disturbed by the treatment. The first reaction of a leatherjacket to disturbance is to contract longitudinally to a squat cigar shape. The second reaction is to extend the body and curl up until the head and anal papillae are apposed. The ventral surface of the body is innermost. Curling and uncurling follow in rapid succession, the rapidity increasing with temperature and severity of stimulation. The third reaction, to extreme stimulation, is to curl up as before and then, with head and tail remaining apposed, revolve the body on its own axis. The result is reminiscent of a smoke ring. The weighing and handling necessary to the observations given below seldom evoked the second reaction, never the third.

RATE OF GROWTH

Groups of larvae

The larval stage may last for as little as 4—5 weeks and as much as 16—17 weeks. This extremely wide variation (with a mean of 8.8 weeks) was encountered among 887 leatherjackets cultured in 37 groups. Table I shows the numbers of pupae produced each week. The start of pupation (i.e. the week in which the first pupae appeared) ranged from the 5th (Group *i*) to the 11th week (Groups *b* & *c*). Within the groups the number of weeks between the appearance of the first and last pupae (pupation spread) varied between 3 and 11. There is no indication that the start and the spread of pupation are correlated. In other words an early or a late start to pupation can be followed by a long or short spread in either case. The distribution of pupation with age is markedly skewed: 90% of pupae appeared by 11—12 weeks and the last 10% took another 5 weeks to arrive. It is often noticeable in individual groups that most of the larvae pupate within 2 or 3 weeks, leaving a few leatherjackets which hang on for a long time, relatively inactive, neither growing nor shrinking until finally pupating. Groups *ff*, *jj* and *kk* were of this type.

There are four larval instars. The first three last an average of a week to 10 days each and the fourth 3 weeks or more. Data from groups of larvae show that the fourth instar may be reached in as little as 3 weeks (Table II). As with the length of the total growth period, there is considerable variation between groups and between individuals. For example, at 3 weeks group *q* contained mainly fourth instars, group *ff* mainly second instars while in group *hh*, second, third and fourth instar larvae were present. However, groups of larvae almost invariably consist of fourth instar larvae only at 6 weeks (for example, groups *r* and *ff* contained 33 and 79 fourth instars respectively at 6 weeks). It is clear

TABLE I

Pupae produced each week by groups of larvae

	Age in Weeks													
Group	5	6	7	8	9	10	11	12	13	14	15	16	17	Totals
a						3	10	21						34
b							3	6	6	0	2			17
c							2	8	7	5	1			23
d					5	10	17	9	2					43
e		1	15	14	10	9	1							50
f						7	7	2	1					17
g					2	6	12	1						21
h			9	6	4	3	1							23
i	1	7	4	4	1									17
j		2	4	16	8	6	4							40
k						13	18	3	6					40
l		14	7	3										24
m			15	10	3	0	1							29
n		1	13	12	5	5	3							39
o				18	15	1								34
p					11	3	1							15
q		5	8	5	1	2								21
r				5	3	5	1	0	0	3	0	1		18
s			6	5	6									17
t			5	4	3	0	1	1	6	3	2	0	1	26
u			9	4	5	0	1	1						20
v		1	1	2	6	3	3	3	3	2	0	1		25
w			1	2	10	6	3							22
x			1	3	5	3	2	0	1					15
y				6	6	4	5	1						22
z				6	5	1	2	0	0	1	1			16
aa				3	7	3	4	3	1					21
bb			2	2	4	5	0	0	1					14
cc				4	7	4	4	1	2	1				23
dd			3	11	5	1	1							21
ee			1	5	11	6								23
ff				3	4	5	2	0	1	1				16
gg		2	2	3	1									8
hh		1	6	16	4	1								28
ii			6	4	13	4	2	1						30
jj			2	13	2	0	1	0	1					19
kk		2	7	3	1	1	1	0	1					16
Totals	1	36	127	192	173	120	113	61	39	16	6	2	1	887

TABLE II

Instar length in groups of larvae

Age of larvae (days)	14		21			28			35		42	
Instar	2	3	2	3	4	2	3	4	3	4	4	Group
Numbers of larvae				15	35		2	36		31		q
							23	15			33	r
										64		ee
			9*	2		2	104	20	21	78	79	ff
	22	63		48	31			61				hh
				33	6		17	21		34		ii
				20	8		4	24		28		jj
				25	4		2	21		20		kk

*On this occasion a sample of 11 larvae only was counted.

TABLE III

Growth of individual larvae up to the third moult

Age (days)	Time* of day	**Weights of larvae (mg) 1	2	3	4	5	6	7	8	9	10
0	12.00	0.12	0.08	0.10	0.06	0.10	0.10	0.10	0.10	0.08	0.10
1											
2	10.00	0.22	0.16	0.18	0.16	0.22	0.20	0.18	0.18	0.18	0.24
3	16.00										
4	10.00	0.50	0.30	0.38	0.42	0.50	0.40	0.44	0.40	0.34	0.50
5	10.00										
6	10.00	1.02	0.50	0.66	0.84	0.84	0.54	0.66	0.66	0.38	0.82
7	11.00										
8	11.00	1.20	0.56	0.90	0.94	0.92	0.82	0.72	0.70	0.44	1.28
9	10.00	1.68	0.56	0.94	0.98	0.94	0.88	0.76	0.70	0.60	1.76
	17.00						0.86				
10	9.00	2.18	0.84	1.06	1.64	0.94	1.20	1.26	0.98	0.76	2.44
11	10.00			0.98							
12	9.00	4.16	1.10	1.32	3.54	dead	2.36	2.68	1.56	1.24	3.76
13	9.00										
14	9.00	6.58	1.26	2.22	5.38		3.88	4.36	2.14	1.00	4.52
15	11.00									1.06	
16	10.00	7.68	2.66	3.70	8.30		7.16	5.46	3.46	1.16	7.28
17	9.00	7.70						6.66			
18	9.00	10.6	4.94	5.56	9.32		9.10	10.2	5.02	1.40	8.36
19	11.00				14.0						
20	15.00	21.8	7.0	7.6	20.4		18.0	17.8	5.6	1.8	16.8
21	11.00										
22	15.00	31.4	10.4	11.6	38.4		31.4	32.2	12.4	2.2	30.0
23											
24	9.00	44.6	19.8	19.4	55.8		48.0	45.0	20.8	3.0	45.4
25	9.00										
26	11.00	48.4	37.6	33.2	68.0		48.4	49.8	32.4	4.8	51.4
27	11.00				75.8						65.6
28	16.00	60.8	55.8	55.4					37.6	7.4	
29			***						***		
30	11.00		63.0	57.2					45.4	7.2	
31	9.00			62.0						9.2	

* On days with no entry in this column, the tubes were not inspected.

** Where the weight is given to 2 decimal places, larvae were weighed on a 10 mg torsion balance; otherwise a 100 mg balance was used.

*** No inspection on 29th day but the larvae were obviously freshly moulted on the 30th day.

Horizontal lines indicate moults — 1 line for 1st moult, 2 for 2nd, 3 for 3rd. Weights for the 3rd instar of larva No. 9 are omitted.

that the wide variation in total growth period arises mainly in the fourth instar.

Of the 887 pupae, 456 (51.4%) were females. On this evidence the sex ratio is obviously unity. Males pupate about a week before females, the means being 8.3 weeks (males) and 9.2 weeks (females). The skewed distribution mentioned above is shown in both sexes.

Individual larvae (first 3 instars)

Ten larvae kept in individual tubes, inspected daily and weighed every other day, gave the data of Table III. The eggs hatched in the 24 hours preceding 'Age O'. Thus age is counted from the start of feeding. Six of the larvae behaved similarly and can be treated as a group. Numbers 2, 5, 8 and 9 were aberrant and are considered individually below. Here it is enough to say that, while numbers 5 and 9 showed signs of abnormality, the other two larvae were rejected because of irregularities in the growth curves which seemed to set them apart from the 6 'typical' larvae.

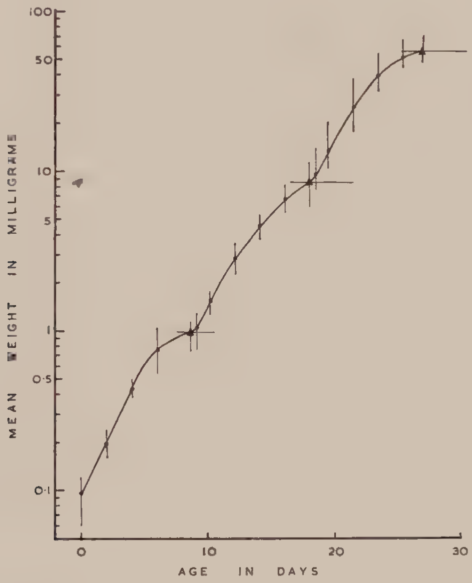

Figure 1. Growth of 6 larvae through the first three instars.

Figure 1 shows the increase in mean weight of the 6 'typical' larvae through the first 3 instars. Weight is plotted on a logarithmic scale. The triangles indicate both mean weight and mean age at moulting. Each vertical line shows the range covered by the individual weights. The horizontal lines show the range of individual age at the moults. The mean weight values for the 2nd and 3rd instars have been estimated from the 6 individual curves using the initial moult as a datum line for each instar. In other words Figure 1 is composed of 3 separate growth curves, one for each instar, the curves for the second and third starting from the average age at the initial and second moult, respectively. The curve shown is quite similar to the more conventional age/mean weight curve (i.e. disregarding the moults) but provides a truer picture in that the slowing down of growth at the moult is less obscured.

The 10 larvae grew from about 0.1 mg to about 60 mg in 27 days. On average the larvae doubled their weight every 3—4 days. The growth rate was highest at the beginnings of the instars, the weight doubling in 2 to 2.5 days.

TABLE IV

Lengths of instars of individual larvae

Larva	Length of instar (days)		
	1st	2nd	3rd
1	7	9	11
(2	13	8	8)
3	10	11	9
4	8	10	8
(5	8	died	)
6	9	8	8
7	8	8	9
(8	8	11	10)
(9	14	16	9)
10	7	10	9
*Mean	8.2	9.3	9.0
*Range	7—10	8—11	8—11

*Mean and Range are given for the 6 'typical' larvae (see text).

There appears to be no correlation between the duration of successive instars in any one larva (Table IV). A larva which takes a long time to reach the first moult does not necessarily grow slowly through the second and third instars as well. Thus the variation in individual ages at the moults is little greater at the 3rd moult than at the 1st (see Figure 1).

The performance of these larvae in tubes was different to that of 50 larvae from the same batch of eggs kept in a group. Growth in the group was faster (Table V). The larvae in tubes lag behind those in the box considerably. The lag starts in the first instar and increases throughout the growth period. At

TABLE V

Comparison of growth rate (as shown by the proportion of larvae of different instars) between individual larvae in tubes and a group in one box (all from one batch of eggs)

	Numbers of larvae							
	in tubes				in the box			
	1st instar	2nd instar	3rd instar	4th instar	1st instar	2nd instar	3rd instar	4th instar
at 10 days	3	7			5	44		
at 17 days		7	2			2	45	
at 24 days		1	8				10	36
at 31 days			1	8				41

24 days the 3rd moult is in progress in the group while the tube larvae are in the middle of the third instar.

The aberrant larvae (numbers 2, 5, 8 and 9) behaved as follows: number 9 grew normally for 4 days, slowly for 4 days, then fairly normally until the end of the instar at 14 days (the drop in weight at the moult was unusually large and the period of no increase in weight was protracted). Coincident with the period of slow growth in the middle of the instar, 2 black spots appeared on the cuticle of the 7th abdominal segment. One of these spots disappeared at the first moult, the other at the second. The time spent in the 3rd instar was normal and it seems likely that the disease or injury which caused the spots also caused the slow growth.

Number 5 died after 3 days in the second instar. It failed to shed the first instar head capsule though the rest of the cuticle was discarded normally (a normal moult may leave the head capsule attached to the body cuticle by a very thin strand or may leave the two parts separate).

Number 2 was apparently healthy, no less active than the six typical larvae and, as its times in the 2nd and 3rd instars show, quite capable of normal growth. Indeed this larva made up for lost time in the second and third instars so that its third moult occurred in the same period as those of the typical larvae. In the first instar it suffered a period of slow growth (from the 6th to the 9th days) though, unlike number 9, without any visible signs of injury or disease.

Number 8 was an apparently normal larva except for its performance in the 2nd instar. It was the lightest of the larvae at the first and the second moults. It grew slowly and moulted late giving a growth curve which was clearly outside the range covered by the 'typical' larvae.

The curve of Figure 1 is offered as a picture of the growth of the typical larva. The exclusion of numbers 5 and 9 needs no justification since one was injured or diseased and one died. The exclusion of numbers 2 and 8 does perhaps require explanation. The growth curve of number 2 in the 1st instar was obviously unlike the curves for the 6 typical larvae. Whereas the latter increased in weight without interruption, No. 2 had a period of 3 days in the middle of the instar when its weight only increased by 0.06 mg. To include this larva in the mean would have given a curve whose shape was representative neither of the typical larvae nor of number 2.

The case for exclusion of No. 8 is weaker. There was no period of delay in the 2nd instar, the growth curve being as smooth as those of the typical larvae. However, the weight at the beginning of the instar was low and the growth rate was also low. These combined to give a growth curve set well apart from the others (the distance from the nearest of the 'typical' individual curves was about equal to the entire range of the 6 larvae). To include No. 8 would have lowered the average curve by about 5% on the weight scale.

Growth of the typical larvae in the first 3 instars is fast and, for the first two thirds of each instar, approximately exponential. Growth slows as the moult approaches but is immediately resumed at a high rate once the cuticle is cast. This is not well shown in Figure 1 but can be clearly seen from part of the data of Table III (larva number 6). This larva moulted between 10 a.m. and 5 p.m. on the 9th day and weighed about the same (0.86 and 0.88 mg) on both occasions.

TABLE VI

Growth of individual larvae in the 4th instar

Weights of larvae (mg)

Days after 3rd moult.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0	105	69	70	90		59	59	51	62	89	81	113	91	68	64	34	60	99	34	83
1					68															
5								98				166				69				147
6			194	271																
7							178				75								117	
8		190												222	206			255		
9						204				132							156			
10	250								159											
11								122				234	277			121				273
12			324	335	185		254													
13											70								180	
14		246												307	288			399		
15						242				213							231			
16	331							138	196			311				137				310
17			396	414									369							
18					203		249				+								201	
19														305	305			442		
20		219				144		153		280		353				174	259			378
21	333		446	488					201											
22							233	+					387						206	
23		141			124		***							221	288			510		
24							141			316		378				168	180		***	394
25	169		451	487					190					168			147			
26													284						116	
27									***			391			236	180		431		453
28				508	466					322		391	215		183					

29									115									***		
30												419				176		238		455
31			465	478						302										
32			+																	
33												378				160				469
34				388						311						***				
36												388				106				466
37				395						340		***								
38												237								
39																				449
40				378						342										
43										330										
44				***																494
45				+																
47																				469
48										332										
50																				455
51										257										
52										202										
53																				444
57																				438
60																				420
61																				***
62																				253
Sex	♀	♂	♀?	♀?	♂	♂	♂	♂?	♂	♀	?	♀	♀	♂	♂	♂	♂	♀	♂	♀

Pupal Weights underlined.

***indicates that the tube was examined and a live larva found.

+indicates that a dead larva was found.

By 9 a.m. the next morning its weight had increased to 1.20 mg, a rate of increase as great as or greater than the rate over the subsequent two days (i.e. weight on 12th day 2.36 mg).

Individual larvae (4th instar)

To study growth in the fourth instar, 20 larvae were cultured singly in tubes. The sand was drained well and the tubes left out of water. Table VI gives the data obtained. 10 male and 6 female pupae were produced. Of the 4 larvae which died, 2 were probably females, 1 was probably a male and 1 died too soon after the moult for its sex to be determined. Sex determination is possible since the male and female growth curves differ significantly from each other. If a larva dies late in the fourth instar its growth curve usually gives a clue to its sex.

Of the 16 larvae which pupated, 3 (1 male and 2 females) showed a delay in growth. Their growth curves are discussed below (p. 195).

The 9 male and 4 female 'typical' larvae gave the curves of Figure 2. Individual weights at the various ages were read off the individual growth curves, to give the mean weights as plotted. The mean pupal weights (i.e. male and female) are plotted at the mean age for pupation and the ranges of 'ages' (i.e. days after 3rd moult) at pupation are shown by the horizontal lines. The range of individual pupal weights is shown by a vertical line.

Each curve in Figure 2 is drawn through two sets of values. For the first part of each curve (crosses) the mean weights are averages of individual weights at 3 day intervals after the moult. For the last part of each curve (dots) the mean weights are derived from the individual weights at 1, 2, 3, 4, 6, 8 and 10 days before pupation. This method gives a better picture of the weight changes taking place. If the initial moult is used as a datum line throughout, mean weights have very little significance after about the 18th day since individual weight changes become erratic. Some larvae are at peak weight, some are pupating, some are losing weight rapidly and so on. The larvae get out of step with each other and an average weight means very little.

The male weight is 60—70% of the female weight throughout the instar. The rate of increase of weight (mg/day) is roughly constant for the first half of the instar and is the same for both males and females. In the second half of the instar the weight rises more and more slowly until peak weight is reached, about a week before pupation in both sexes. In the last week the weight falls by 40—50%.

Probably most of this loss in weight is due to the expenditure of reserves involved in the formation of the pupa. Food in the gut of a larva at the peak only accounts for about 10% of its weight. The cast larval skin weighs about 20 mg, accounting for a further 5—10%.

Further illustration of the pre-pupation drop in weight is provided by the individual curves for 3 larvae (numbers 7, 12 and 15). The pupae were soft and white when discovered. Pupae only stay white for an hour or two after pupation so the time of moulting is precisely known. Number 7 lost 92 mg in the 2 days before pupation and 16 mg in the 4 days before that. Number 15 was weighed 1 day before pupation and lost 53 mg during the 24 hour period. Over the previous 4 days it had lost 52 mg. Number 12 (a female) lost 101 mg in the 48 hours before pupating and 40 mg in the 3 days before that. The mean weight curves for males and females show smaller losses in the last two days before pupation and higher losses in the preceding 4 days. In other words the drop in weight is even more rapid than the curves in Figure 2 show.

Figure 2 indicates the type of growth exhibited by the majority of 4th instar larvae: fast growth at a constant rate (i.e. a constant daily weight increase) for the first third to a half of the instar; then a decline in growth rate to nothing over the space of a few days leaving the larval weight at its peak. Peak weight is maintained for at most a week in individual larvae before the drop to pupation begins. As mentioned on p. 194, 3 larvae (Nos. 10, 16 and 20, see Table VI) did not conform to this pattern. The male pupated 35 days after the 3rd moult (other males ranged from 18 to 28.5 days) and the females pupated at 52 and 62 days respectively (other females 23.5 to 38.5 days).

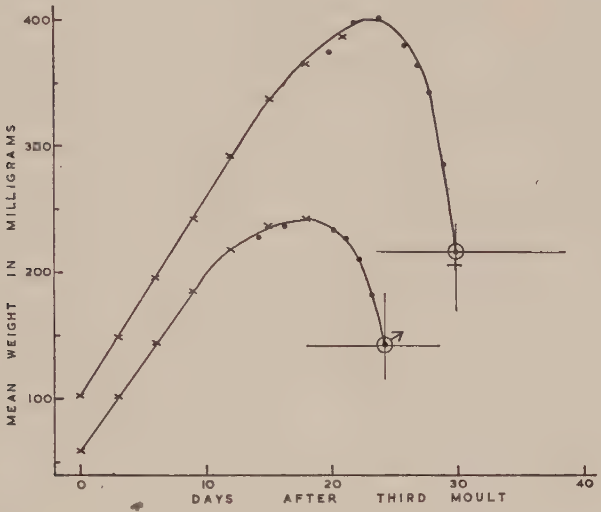

Figure 2. Growth of 13 larvae (9 ♂, 4 ♀) through the fourth instar.

The 3 larvae are peculiar, not only with respect to the length of instar, but also in the shape of the growth curve. Both the females grew as fast as the 'normal' females for the first 25 days of the instar. Having reached the peak however, they stayed at that level, the weight sometimes rising and falling by as much as 40 mg, for 24 days (No. 10) and 30 days (No. 20) respectively. The drop in weight before pupation appeared perfectly normal and the resulting pupae and adults showed no deformities. The male (No. 16) grew more slowly than the typical males, reaching peak weight in 20 days (typical males 10—15 days). It then stayed at this level for about 12 days (typical males, about 1 week), at last pupating normally at 36 days. The typical males pupated between 18.0 and 28.5 days so the delay in this case was not very large. Nevertheless it was very obvious that this larva was 'hanging around' at peak weight unlike the typical larvae which show a relatively smooth rise and fall in weight before and after the peak.

DISCUSSION

Larval growth in *Tipula oleracea* follows a course which is common among insects: 1. exponential growth throughout the early instars giving a linear relationship between log. weight and time, interrupted, of course, towards the ends of the instars. 2. a constant daily weight increase for the first part of the last instar (a linear relationship between weight and time). The curve of weight increase is of the same type in the larva of the garden chafer *Phyllopertha horticola* L. (Col. Scarabeidae). It has been suggested (LAUGHLIN, 1957) that in the early instars the larva grows in all parts of the body. Every organ is increasing in size, internal organs day by day, the cuticle in steps at the moults. Thus the rate of weight increase rises with rising weight. By the time of the last larval moult all the organs except the fat body have reached full size and the principal purpose of last instar feeding is to pack away reserve substances for metamorphosis and reproduction. The maximum speed of this process is governed by the size and activity of organs of assimilation. Since these have attained maximum size, food storage (and hence body weight increase) goes on at a constant rate of so many milligrams per day.

The difference in growth rate, between larvae isolated in tubes and larvae in a group, (see p. 190) may be due to a number of causes. The tube larvae were handled more frequently, also cleaned out and fed more frequently than the box larvae. Feeding and moisture conditions may have been slightly different.

Another possibility is that isolation itself slows growth. IWAO (1956 and 1958) showed that larval growth in the armyworm (*Leucania unipuncta* Haworth) varied significantly with variation in culture density. The migratory locust, (*Locusta migratoria migratorioides* R. & F.) also exhibits a number of physiological effects of rearing density (see, for example, ALBRECHT, VERDIER & BLACKITH, 1958). Both of these species, of course, have definite behaviour patterns dependent on population-density and might be expected to show associated physiological changes. Larval migrations have been reported of various crane flies but no relation with larval density has been established. The situation might well repay further investigation.

The delayed pupation described on p. 195 is a recurring phenomenon in *T. oleracea.* The skewness of the distribution of age of pupation (p. 186) is due to extension of the time spent at peak weight more than variation in rates of increase in weight. Delayed pupae have been produced in many more groups than are cited here and under a variety of conditions. It is probable in fact, that individuals showing delayed pupation occur in the field and are not simply an artefact of the culture method.

I would like to thank Miss R. F. Potter for technical assistance with the cultures and Dr. A. Milne for constructive criticism of this paper.

ZUSAMMENFASSUNG

DIE BIOLOGIE VON TIPULA OLERACEA *L.: DAS WACHSTUM DER LARVE*

Die Larvalentwicklung von *T. oleracea* kann unter konstanten Bedingungen bei 21° C 4—5 Wochen, aber auch 16—17 Wochen dauern (— die durchschnittliche Dauer für 887 Larven, die in 37 Gruppen gezüchtet wurden, betrug 8,8 Wochen —). Der Gipfel der Entwicklungszeitkurve liegt innerhalb des ersten Abschnittes der Kurve, indem sich 90% der Larven zwischen dem 30. und dem 80. Tag verpuppen.

Die ersten drei Larvalstadien werden in 3—6 Wochen durchlaufen; selten in längerer Zeit. Die großen Unterschiede in der Dauer der Larvalentwicklung entstehen daher vor allem im 4. Stadium. Larven in Gruppen entwickeln sich schneller als isolierte Larven.

Männliche Larven verpuppen sich ungefähr eine Woche vor den weiblichen Larven. Das Zahlenverhältnis zwischen männlichen und weiblichen Larven beträgt 1. Beide Geschlechter ergeben für die Dauer der Larvalentwicklung die gleiche schiefe Häufigkeitsverteilung.

Das Gewicht vergrößert sich in den ersten drei Larvalstadien jeweils während der ersten zwei Drittel potentiell. Im Durchschnitt verdoppeln die Larven während der Gesamtdauer der ersten drei Larvenstadien ihr Gewicht alle 3—4 Tage. Während des ersten Abschnittes des letzten Stadiums vergrößert sich jedoch das Gewicht um den festen Betrag vom 15 mg pro Tag. Es erreicht seinen Höhepunkt und nimmt dann schnell ab, wenn sich in der letzten Woche der Darm entleert und die Puppe sich bildet. Das Gewicht der Puppe beträgt etwa die Hälfte des Höchstgewichtes der Larve.

Einige Larven erreichen ihr Höchstgewicht, verlieren aber erst nach 4 Wochen an Gewicht und verpuppen sich dann. Diese Ausnahmefälle sind der Hauptgrund für die oben erwähnte Schiefe der Häufigkeitsverteilung.

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THE REACTION OF MOSQUITOES TO MOISTURE AND HIGH HUMIDITY

BY

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A method is described by which the effect of a wet surface and the air close to it (up to a height of 2 cm) on female *Aedes aegypti* (L.) could be determined. The insects were placed in a petri dish, under controlled conditions of ambient temperature and humidity. The bottom of the petri dish was lined with two semicircular filter papers, one being kept dry and the other wet. The intensity of the response was determined as the mean excess percentage of position records on the dry and wet sides.

Female mosquitoes avoided very strongly a wet surface and moderately the air close to it.

Mosquitoes did not feed on a human arm as long as the skin was kept wet.

Desiccated mosquitoes (water loss of 5.6% or higher of their body weight) were strongly attracted to a wet surface and moderately to the air close to it. The higher the desiccation, the stronger was the attraction.

Gravid mosquitoes moderately avoided a wet surface, but gave no reaction to the air close to it. There is some evidence that gravid mosquitoes must get into a "laying state" before attraction to a wet surface occurs.

The higher the ambient R.H., the stronger the avoidance of a wet surface and of the air close to it.

High and low ambient temperatures (35 and 18° C) gave a weaker dry response than a medium temperature (28° C).

The causes of the contradictory results obtained by various investigators reporting the response of mosquitoes to moisture are discussed. These differences are most probably due to differences in the technique used and in the physiological state of the mosquitoes.

The avoiding reaction from a wet region is due to a taxis-type, not to a kinesis-type, reaction.

Reports on the reaction of mosquitoes to moisture or high humidity are contradictory, even when they apply to the same species. CHRISTOPHERS (1947), working at 25° C and 80—90% relative humidity (R.H.), found female *Aedes aegypti* to be unaffected by a moist surface. PARKER (1948), working on the same insect at 28° C and 50—70% R.H., found that a moist surface exerted quite a definite attraction. In order to test if the difference in ambient temperature and humidity were the reasons for this contradiction, PARKER (1952), in another series of experiments, came to the conclusion that the temperature and humidity differences, operating at the time of the experiment, could account for part but not all of the discrepancy. BROWN (1951) showed that the surface moisture of a clothed robot increased its attractiveness to various *Aedes* species 2 to 4 times, provided the air temperature exceeded 60° F. PLATT, COLLINS & WITHERSPOON (1957) found that *Anopheles quadrimaculatus* avoided high as well as low humidities (the optimum was between 70—80% R.H.). These reactions were independent of temperature, light intensity and colour. Similar examples, of mosquitoes avoiding high as well as low humidities, were reported by RUDOLFS (1923, 1925) on *Aedes solicitans, A. cantator* and *Culex pipiens*, THOMSON (1938) on *Culex fatigans*, ROTH & WILLIS (1952) on *A. aegypti*. BROWN, SARKARIA & THOMPSON (1951) found that a moistened billiard-ball was approximately seven times as attractive to *A. aegypti* as a dry one, the R.H. was 30—40%. When the comparison was repeated at a R.H. of 80% the results were reversed, the dry ball being 2.5 times as attractive as the moist ball. They also found moist air (85% R.H.) to attract 3—5 times as many *A. aegypti* as dry air (15% R. H.). SMART & BROWN (1956) observed that hands which perspired the most moisture were the least attractive. The authors found it "surprising" since previous results (BROWN et al., 1951) showed a moist stream of air to be more attractive than a dry one. In experiments with *Anopheles maculipennis* and *Aedes aegypti* MARTINI & TEUBNER (1933) found that both species showed a general preference for higher humidities although the reaction was by no means always sharp. REUTER (1936) showed that moisture was distinctly attractive to *A. aegypti*. DE LONG, DAVIDSON, PEFFLY & VENARD (1945) found moistened warm air more attractive to *A. aegypti* than warm air. DE MEILLON (1937) concluded that *Anopheles gambiae* and *A. funestus* would prefer a more humid air if they had been starved for 24 hours, or maintained for some hours at a low humidity, but there was no significant reaction if they had fed on sugar water or blood. LAARMAN (1958) collected evidence that heat and moisture release alighting of *Anopheles atroparvus* on the host. LEWIS (1933) and BAR-ZEEV (1957) concluded from experiments on *Aedes aegypti* that the length of life of the mosquitoes is dependent on humidity.

The original purpose of the present investigation was to test the effect, from a behavioral point of view, of various materials on the contact chemoreceptors of the tarsi of mosquitoes. Since some materials were to be used in aqueous solution, the effect of a wet surface and the air close to it was first to be determined, especially in view of the contradictory results obtained by various investigators as reported above.

MATERIALS AND METHODS

The method was as follows: The mosquitoes were confined in a petri dish (15 cm in diameter, 2 cm high) covered with mosquito-gauze. The bottom of the petri dish was lined with two semicircular filter papers (Whatman No. 1) made of two thicknesses. One semicircular filter paper was kept dry while the other was moistened with 2½ ml of distilled water. A neutral band, 1 cm wide, along the midline, covered with adhesive tape, separated the dry and wet filter papers. The petri dish, containing the mosquitoes, was placed in a closed container, the temperature and humidity of which could be controlled. The position of the mosquitoes could be observed from the outside (details of the apparatus will be given later). For determining the effect of the moist surface, counts were taken every minute of mosquitoes standing on the dry and wet filter papers. Those standing, partly or wholly, on the neutral midline or above the filter papers (mosquito-gauze, walls of the petri dish) were ignored. After every second count the petri dish was jarred, by means of a lever, to change the position of the mosquitoes. After 20 counts the petri dish was turned 180°, to counteract any bias for a particular side, and another 20 counts, as previously, were taken. In each such test 12 mosquitoes were used; the test was repeated with different mosquitoes, six or more times, so that each set of experiments consisted of at least 240 counts. For determining the effect close to the wet surface (up to a distance of 2 cm; the height of the petri dish), a double stainless steel screen, 1 mm thick, soldered at the edge, covered both semicircular filter papers, fitting exactly the bottom of the petri dish. This prevented any contact of the mosquitoes with the filter papers. Counts were taken, as previously, of mosquitoes standing above the dry and wet filter papers, either on the screen or above it (mosquito-gauze and wall of the petri dish). Those standing partly or wholly above the neutral midline were ignored. The index of reaction (I.R.), a measure of the intensity of the response to wetness or high humidity (as the case may be), is the mean excess percentage of positions records on the dry or wet side. The I.R. for each test was calculated from the formula 100 (D—W)/N (GUNN & COSWAY, 1938), where D = number of mosquitoes on the dry filter paper, or in the case that a screen was used, on the dry half, W = number on the wet filter paper, or wet half, N = total number on both dry and wet filter papers, or on both halves. An I.R. of 0 indicates no preference to either side. A positive value and its magnitude shows preference to the dry side and its relative preference (the maximum is + 100). Similarly, a negative value and its magnitude shows preference to the wet side and its relative preference (the maximum is — 100). In a set of experiments, twice the standard error indicates that a significant reaction has taken place.

"Mosquitoes" will designate, unless otherwise specified, female *Aedes aegypti*, 4—5 days old, which had water and honey at their disposal prior to the experiment, and never had a blood meal (non-gravid).

The following terms will be used: "a dry reaction" will mean avoidance of a wet surface, "a wet reaction" — attraction to a wet surface.

Details of the apparatus used

Details of the apparatus in which counts of the mosquitoes were taken under controlled conditions of temperature and humidity (Fig. 1) are as follows: The petri dish, containing the mosquitoes, was inserted into the ring of a tripod, situated on top of a box (42 x 42 x 8 cm). The tripod was covered with an inverted jar (21 cm in diameter, 21 cm high), made of perspex, which could be tightened to the box, by a circular rubber sponge, to make it air tight. The jar was then covered with an incubator, which consisted of a bottomless wooden box (35 × 35 × 20 cm), having a circular opening at the top, lined with rubber, which fitted tightly the top of the inverted jar through which the mosquitoes in the petri dish could be observed. The temperature in the box was controlled by a bimetallic thermostat, thus controlling also the temperature inside the jar. The top of the inverted jar was covered with a metal ring (17.5 cm inside diameter, 6.5 cm high), covered with a heating mantle, and which could be heated to any desired temperature by means of a rheostat. The purpose of the ring was to keep the top of the inverted jar warm to prevent vapour condensation on it, when the room temperature was below the temperature inside the jar. A constant flow of air (8 liters per min.) passed through a flowmeter into a tubing coil immersed in a water bath at controlled temperature. From there, the air bubbled through gas washing

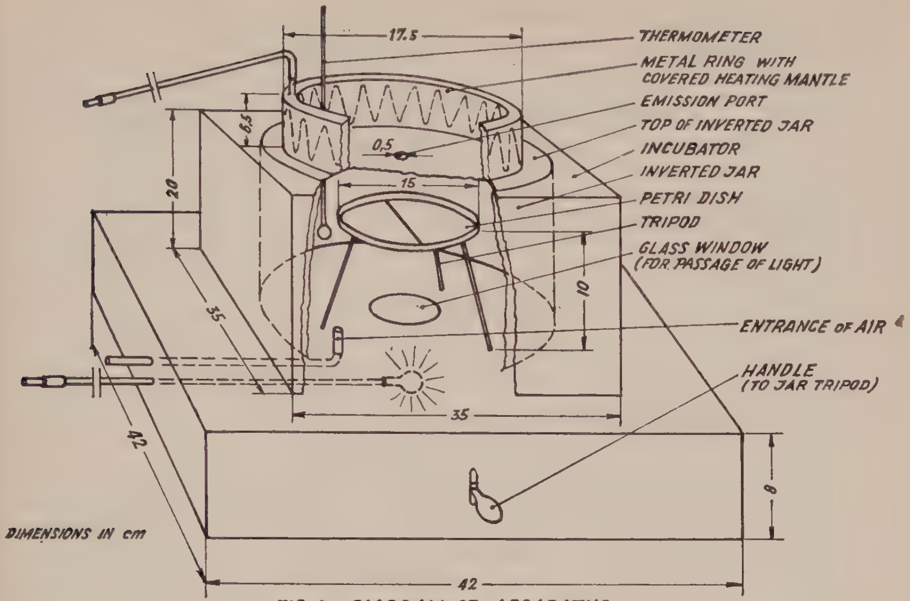

FIG I. DIAGRAM OF APPARATUS

bottles immersed in the same water bath, and containing a suitable saturated salt solution to obtain the required R.H. The air then passed into the inverted jar at the bottom, below the centre of the petri dish, and out through an orifice (0.5 cm in diameter) at the centre of the top of the inverted jar.

In most of the experiments 61% R.H. was used; the air bubbled through a saturated solution of $NaNO_2$. In other experiments very low (25%) and very high R.H. (95%) were also used. The very low R.H. was obtained by letting the air pass through gas washing bottles containing $CaCl_2$. The very high R.H. was obtained by letting the air bubble through water at 35° C, while the temperature inside the jar was kept at 26° C. The above mentioned R.H. were determined by the dry and wet bulb method. The air from the jar passed the wet bulb thermometer at a speed of not less than 2 m/sec.

Further accessories were a lever so connected to one leg of the tripod that by tapping on the lever, it jarred the tripod with the petri dish on it. At the bottom of the apparatus, opposite the centre of the petri dish, was a circular glass window (8 cm in diameter) through which light from a small electric bulb illuminated the floor of the petri dish. A thermometer passed through the top of the inverted jar with its bulb brought close to the edge of the petri dish.

RESPONSE TO HUMIDITY AND MOISTURE

Effect of a wet surface and the air close to it

Unless otherwise stated all experiments were carried out at an ambient temperature of 26 ± 1° C and an R.H. of 61%. In the following experiments the reaction of the mosquitoes to a wet surface and to the air close to it was determined.

Control experiments, with both surfaces dry, where also carried out. Results are given in Table I.

TABLE I

The reaction of the mosquitoes to a wet surface and to the air close to it

Type of surface	Number of replicates	N*	I.R. ± S.E.**
	Close to the surface		
Dry and wet	6	2711	41.6 ± 3.2
Dry and dry (control)	6	2693	3.6 ± 2.4
	On the surface		
Dry and wet	12	1761	92.1 ± 1.5
Dry and dry (control)	12	2553	—3.5 ± 3.9

* N = Total number of mosquitoes counted

** I.R. ± S.E. = Index of reaction ± standard error (see text)

It can be seen that the mosquitoes gave a very strong dry reaction on the surface and a moderate dry reaction close to it. In controls the I.R. was relatively close to the theoretical value for no reaction (0.0%).

The effect of a temperature difference at the dry and wet surfaces due to evaporation

The temperature at the wet surface is lower, due to evaporation, than at the dry surface. This difference, at 26° C, was found to be, at 1 mm above the surface, about 1.5° C. In order to determine if this difference in temperature affects the I.R., the following experiments were carried out. Two cut halves, of a similar petri dish, were joined together with adhesive tape along the median line, leaving an air space of 0.5 cm between the two halves. The wet half was placed on an electric plate, the temperature of which could be controlled, and so adjusted that the temperature on both sides (dry and wet) was equal. The I.R. obtained, under these conditions, on the surface and close to it were similar to the results (dry *versus* wet) given in Table I. The small difference in temperature of a wet and dry surface had, therefore, no effect on the I.R.

Effect of lowering the R.H. close to the wet surface

Since the avoiding reaction close to the wet surface was due to the very high R.H., it was interesting to know whether the mosquitoes still avoid a wet region if the humidity is lowered somewhat. For this purpose similar tests were carried out as previously, with a screen, but instead of wetting the semicircular filter paper with distilled water, it was wetted with 2½ ml of a saturated solution of zinc sulfate. Such a solution gives a R.H. of 90% in a closed vessel at 20° C. The I.R. close to the wet surface, thus obtained, was 15.7 ± 3.4 (10 replicates, N = 4384). Lowering the R.H. by about 10% close to wet surface still produced a dry reaction; however, this was greatly reduced (almost 3 times) as compared to the reaction when distilled water was used (Table I).

Effect of different ambient R.H.

The effect of the ambient R.H. on the reaction of the mosquitoes to a wet surface and to the air close to it was next determined. The experiments were carried out with very high (95%) and very low R.H. (25%), and the results compared with those obtained with a 61% R.H. (For method of obtaining and determining the various R.H. see "Materials and Methods"). Results are given in Table II.

TABLE II

The reaction of the mosquitoes to a wet surface and to the air close to it at different ambient R.H.

No. of replicates	N	R.H.	I.R.
		Close to the surface	
6	2647	25	12.0 ± 1.1
6	2711	61	41.6 ± 3.2*
6	2726	95	66.5 ± 4.6
		On the surface	
12	2216	25	67.3 ± 4.6
12	1761	61	93.1 ± 1.5*

* Results taken from Table I.

The higher the ambient R.H., the stronger was the dry reaction close to the surface as well as on the surface.

Effect of ambient temperature

In the following experiments the effect of the ambient temperature on the reaction of the mosquitoes to a wet surface and to the air close to it was tested. A high (35° C) and low (18° C) temperature was used, and the results are compared with those obtained previously at 26° C (Table III).

TABLE III

The reaction of the mosquitoes to a wet surface and to the air close to it at various temperatures

Number of replicates	N	Temp. °C	I.R.
		Close to the surface	
6	2605	18	28.6 ± 1.4
6	2711	26	41.6 ± 3.2*
6	2513	35	37.8 ± 5.2
		On the surface	
12	1462	18	51.4 ± 3.9
12	1761	26	93.1 ± 1.5*
6	1760	35	63.2 ± 5.0

* Results taken from Table I.

It can be seen that in spite of the large differences in the temperatures used, there was still a strong to moderate dry reaction on the surface and close to it. But at the extreme temperatures used the dry reaction was significantly weaker (except at 35° C close to the surface) than that at 26° C.

Reaction of desiccated mosquitoes

In the following experiments the mosquitoes were desiccated over $CaCl_2$ in a hermetically sealed desiccator over a period of 1, 2 and 4 hours at 28° C, and their reaction to a wet surface and to the air close to it was determined. The per cent of water loss, as determined by loss of body weight, was found to be 3.2 ± 0.6, 5.6 ± 0.6, and 10.3 ± 0.5 per cent, respectively. To prevent these mosquitoes from drinking water from the wet filter paper during the experiment, the proboscis of each mosquito was removed close to its base, prior to drying, with a fine scalpel. The edge of the remaining part of the proboscis was cemented with a solution of collodion. Results are given in Table IV.

TABLE IV

The reaction of desiccated mosquitoes to a wet surface and to the air close to it

No. of replicates	N	% water loss of body weight	I.R.
		Close to the surface	
6	2476	3.2 ± 0.6	0.0 ± 4.1
6	2686	5.6 ± 0.6	—24.0 ± 2.5
6	2647	10.3 ± 0.5	—30.7 ± 3.4
		On the surface	
6	1379	5.6 ± 0.6	—67.9 ± 6.6

At a water loss of 3.2% of their body weight, the mosquitoes have already lost their dry reaction close to the surface. Further desiccation caused a wet reaction close to the surface, which increased with increased desiccation. On the surface, at a water loss of 5.6% of their body weight, the mosquitoes gave a significantly stronger wet reaction than close to it.

Reaction of gravid mosquitoes

Since these mosquitoes lay eggs on a wet surface, it was of interest to determine the reaction of gravid mosquitoes to a wet surface and close to it. In order to prevent gravid mosquitoes from laying eggs prior to the experiment, they were placed in a pint jar covered with a mosquito-gauze. A V-shaped volumetric pipette filled with water was introduced into the jar. The mosquitoes could drink from the capillary tip of the pipette but were unable to lay eggs. Water was added daily to the pipette by means of a fine capillary tube, filled with water, introduced into the stem of the pipette and reaching the level of the water. A cotton wad soaked in honey was placed on the mosquito-gauze which covered the jar. Under these conditions gravid mosquitoes, not having a suitable wet surface to lay on, could be kept for any desired period of time without laying any eggs.

Preliminary observations have shown that when the mosquitoes were kept at 28° C and 70% R.H., about 20 per cent laid eggs on the second day after a blood meal, about 70 per cent on the third, and about 10 per cent on the fourth. Gravid mosquitoes, used in the following experiments, were 4 or more days after a blood meal, and their oviposition deferred as previously described.

Experiments were carried out, as previously, with and without a screen. In the former case, no eggs were laid on the wet filter paper (through the screen) during the experimental period. In the second case, an average of 26 eggs was laid per test. This is a very small number of eggs considering that each test consisted of 12 females, and the average number of eggs laid in controls was found to be 93/female. It will be recalled that the mosquitoes were "disturbed" mechanically every second minute by jarring the petri dish. But even when undisturbed, gravid mosquitoes were observed to be relatively slow to start egg laying when the opportunity was offered. This will also be seen in further experiments to be reported later. The length of deferment of oviposition did not affect the I.R. Therefore, the results of the various groups tested on different days after a blood meal (4—10 days) were combined. The I.R. close to the surface was 1.3 ± 3.0 (37 replicates; N = 16155), and on the surface 38 ± 5.1 (18 replicates, N = 4613). It shows that gravid mosquitoes did not show any preference close to the surface, whereas on the surface the dry reaction still persisted, but was greatly reduced when compared with non-gravid females (Table I).

Effect of desiccating gravid mosquitoes on the length of time required to start egg laying

Gravid mosquitoes must come at one time or another to a wet surface in order to lay eggs. Inasmuch as gravid mosquitoes did not show a wet reaction on the surface (although the dry reaction was greatly reduced), it was believed that the mosquitoes are attracted to water so as to lay eggs only when they have lost a sufficient amount of water. Once attracted and in contact with water, they are stimulated to lay eggs. In order to test this theory, individual desiccated gravid mosquitoes (4 hours over $CaCl_2$), as well as non-desiccated gravid mosquitoes, were placed in cages (30 x 30 x 30 cm). A small vial (4 cm high, 3½ cm in diameter), containing water and lined with filter paper, was placed in the cage. Every half hour, for a period of 2 hours, the vial was replaced by another. The number of females which laid eggs in the first, second, third and fourth half hours could thus be determined. The experiments were replicated 10 times, using a total of 50 desiccated and the same number of non-desiccated mosquitoes. Results are given in Table V.

TABLE V

The effect of desiccating gravid mosquitoes on the start of oviposition

Time in hours from the start of the experiment	Per cent of total females, laying eggs	
	Desiccated females	Non-desiccated females
½	34.0	22.0
1	42.0	36.0
1½	50.0	52.0
2	62.0	62.0

The per cent of desiccated gravid mosquitoes which laid eggs was somewhat higher in the first half hour. This difference was much smaller after 1 hour and vanished after 1½ and 2 hours. The results obtained do not support the assumption that loss of water may be neccessary to induce the mosquitoes to come to water and to lay eggs. It can also be seen that gravid females, in spite of deferred oviposition, did not start to lay eggs immediately when the opportunity was offered. Only about 50 per cent of the females laid eggs 1½ hours from the start of the experiment. This fact was also observed when gravid mosquitoes of deferred oviposition were placed in a petri dish, the bottom of which was lined with a wet filter paper. More than 50 per cent of the mosquitoes either did not lay eggs, or laid less than 10 eggs/mosquito in one hour observation. It was also observed than once a mosquito started to lay eggs, it would usually continue to do so until all the eggs have been laid (an average of about 90 eggs/mosquito), and would not lay any more eggs until a second blood meal has been obtained. These facts strongly indicate that gravid mosquitoes, when close to or on a wet surface, must probably get into a physiological state of "egg laying", and that it takes some time for this state to be reached. It is probable that only when such a state has been reached are the mosquitoes attracted to a wet surface to oviposit.

The effect of moistening the skin on the biting of mosquitoes.

Since mosquitoes were found to avoid strongly a wet surface, it was of interest to determine how a wet skin will affect their biting. Individual mosquitoes were confined in a glass tube (1.3 cm in diameter, 3 cm in length). One end of the tube was covered with mosquito-gauze, and the other applied to a human arm moistened with distilled water. The tube was held horizontally. Every minute or so, the tube was moved on the skin to another part that was just moistened (unless the mosquito happened to stand on the skin) in order to keep the tube in contact with a wet skin all the time. The experiments were replicated with 15 females. No mosquito fed on the arm as long as the skin was wet. In some cases the mosquito approached the skin, but soon retreated before touching it. In other cases the mosquito may have landed on the wet skin but soon retreated without biting. In controls (without wetting the skin), it took from a few seconds to 3 minutes at the most to start feeding. In another set of experiments 10 females were confined in a glass tube (3 cm in diameter, 15 cm in length) closed at both ends with mosquito-gauze. One end of the mosquito-gauze was moistened with distilled water, and the arm of a human was held tightly to the wet mosquito-gauze for 15 minutes, the tube being held horizontally. The mosquito-gauze was moistened 4 or 5 times to keep it wet. No mosquito fed under these conditions. In control experiments (without wetting the mosquito-gauze) most of the mosquitoes fed within a few minutes. It is thus obvious that a wet skin acted as a repellent to the mosquitoes. These results may explain the lower attraction to perspiring hands (SMART & BROWN, 1956).

MECHANISM OF THE REACTION

In general, orientation of an insect involves klinokinesis, hygrokinesis, orthokinesis and klinotaxis (FRAENKEL & GUNN, 1940). In some insects studied the response to humidity was due only to kinetic-type reaction (KENNEDY, 1937; BENTLEY, 1944; ROTH & WILLIS, 1951b). A taxis-type reaction was observed by THOMSON (1938), whereas a combination of both types of reactions was exhibited by other insects (LEES, 1943, 1948; PIELOU & GUNN, 1940; BURSELL & EWER, 1950; SMEREKA & HODSON, 1959).

In the present experiments, it was observed that when the mosquitoes were given a choice of dry and wet surfaces, most of the mosquitoes were found on or above the dry surface. When the petri dish was jarred, many mosquitoes were flying for a few seconds, and most of them landed again on or above the dry surface. It could often be observed that a mosquito flew back and forth along the sidewall of the dry side of the petri dish, from one end of the midline to the other. This behaviour suggests that klinotaxis is involved, namely a directed reaction. The mosquito compares the intensity of stimulation in its vicinity by alternate movements or by movements of the antennae.

To determine which type of reaction is involved, more detailed experiments were carried out. Individual mosquitoes were placed in a petri dish, under similar conditions as previously. One semicircular filter paper was wet and the other dry. Every minute or so, the petri dish was jarred by means of the lever of the apparatus, to induce the mosquito to fly. A kymograph was used to record the time spent in flight over the dry and wet surfaces. Twelve flights of each of 20 single mosquitoes were thus recorded (a total of 240 flights). The part of the total time in flight, spent over the dry and wet surfaces, was 80 and 20 per cent, respectively. This indicates that a taxis-type rather than a kinesis-type of reaction is involved, since the activity over the wet surface is not greater (rather much lower) than over the dry surface.

In order to determine if hygrokinesis may also be partly involved, individual mosquitoes were placed in a similar petri dish, the bottom of which as well as the walls were lined with moistened filter paper. The mosquito-gauze which covered the petri dish was also moistened. Thus, the mosquito was forced to stand only on a wet surface. As soon as the mosquito was introduced, the length of time of its activity (flying, walking), as well as the frequency of movements were recorded for a period of five minutes by means of a kymograph. At the end of the experimental period, the mosquito was transferred to a similar dry petri dish and its activity, as well as frequency of movements, were similarly recorded. Twenty individual mosquitoes were thus tested. Ten were tested first on a dry then on a wet surface, and ten others first on a wet then on a dry surface, to counteract any possible previous effect. The lengths of time spent in activity over the dry and wet surfaces were similar (20 per cent of the total time). Similar frequencies of movements (a total of about 80 in the 5 minutes tested) were also observed under the dry and wet conditions. It appears, therefore, that hygrokinesis is not involved in the reaction. These results agree with the observations made by THOMSON (1938) on *Culex fatigans*. It must be emphasized that not all the orthokinetic complex is dealt with by present methods; whether the speed of movement is affected by humidity has not been determined.

DISCUSSION

The method used in the present investigation has the advantage that it permits determination of not only the effect of differences in R.H., but also the direct effect of a moist surface. No attempt was made to determine the difference in R.H. between the air close to the dry and wet surface. There is obviously a humidity gradient from the wet surface upwards, and a steep humidity gradient close to each side of the dividing median neutral zone, with a much less steep gradient in the other parts.

In the experiments reported above, the mosquitoes gave an intense dry reaction on the surface and a moderate dry reaction close to it. Ambient temperature and R.H. (especially the latter) had an effect on the degree of the intensity, but the dry reaction still persisted, even when relatively extreme temperatures and humidities were used. This consistency of the dry reaction raises the question why contradictory results were obtained by various investigators on the same species, with regard to the attraction of mosquitoes to moisture. The answer seems to lie in the fact that the results obtained depend mainly on two factors: a) the physiological state (water balance, gravidity) of the mosquitoes, as shown in the present investigation. Other arthropods were also shown, by various investigators, to give a reversed wet reaction, due to a change in their water balance (DE MEILLON, 1937; GUNN & COSWAY, 1938; BENTLEY, 1944; LEES, 1948; WILLIS & ROTH, 1950; ROTH & WILLIS, 1951b; PERTTUNEN, 1955; SMEREKA & HODSON, 1959). b) the technique used in the testing procedure. If the test is carried out in such a way that a relatively large number of mosquitoes in a big cage is used and the numbers of mosquitoes attracted to a wet *versus* a dry surface are compared (BROWN et al., 1951), it is obvious that only a small percentage of the mosquitoes actually participates in the reaction. The results refer, therefore, to a selected group of mosquitoes. Among a large number of mosquitoes, there may be expected to be a certain percentage in which their water balance is such as to attract them to a wet surface. It is suggested that the nonresponsive individuals were repelled. In the present investigation, nearly all the mosquitoes were forced to make a choice between a dry and wet surface or to the air close to it. This is in accord with the results obtained in an open field (BROWN, 1951) with robots, whose clothing had been moistened compared to dry clothing, and which showed that moisture is an attractant factor to several Canadian species of *Aedes* WILLIS & ROTH (1952) showed how contradictory results were obtained, on the effect of CO_2 on *A. aegypti,* when all of the mosquitoes were forced to participate in the reaction in a small cage olfactometer, or part of the mosquitoes (less than 10 per cent) in a large cage olfactometer. They concluded that the reason for this contradiction was that only part of the mosquitoes are attracted to CO_2 at any given time, and the percentage of mosquitoes participating affects, therefore, the results. Another factor involved is the length of time, prior to the experiment, during which the mosquitoes are deprived of water, thereby affecting their water balance, and, as a corollary, the existence of the opportunity to replenish the loss of water by having access to drinking water during the experiment. In the present investigation, the mosquitoes could drink water from the wet filter paper also when a screen was used. They were capable of reaching the wet filter paper by inserting their proboscis through the screen.

It has been shown by REUTER (1936) and BURGESS & BROWN (1957) that blood was more attractive than water. It has already been pointed out by BAR-ZEEV & SMITH (1959) that this result may be due mainly to a difference in the R.H. close to the surface of these materials.

It has been shown (Table II) that the higher the ambient R.H., the stronger was the dry reaction close to the surface. This may be explained by the assumption that the closer to 100% R.H. the more intense the reaction, as observed on various insects (THOMSON, 1938; LEES, 1943; BENTLEY, 1944; WILLIS & ROTH, 1950; SMEREKA & HODSON, 1959). It has also been shown (Table II) that at a very low ambient R.H. (25%) the dry reaction was relatively low (I.R. = 12.0 ± 1.1). As already mentioned, mosquitoes were shown to avoid high as well as low R.H. If this is so, it may be expected that the mosquitoes will also avoid the dry air, thereby causing a weak dry reaction.

It has been shown (Page 202) that when the R.H. close to the wet surface was lowered by about 10 per cent, (by using an appropriate salt solution instead of water) the avoiding response was greatly reduced (I.R. = 15.7 ± 3.4). This may be due to either one or a combination of the following two factors: a) a decrease in R.H. in the more humid alternative (this has been observed to decrease the response of various insects, as already mentioned). b) a smaller difference in the humidity gradient. This has also been shown to decrease the intensity of the reaction of certain insects (KENNEDY, 1937; SMEREKA & HODSON, 1959).

The dry reaction was decreased at relatively extreme temperatures (18 and 35° C) as compared to 26° C (Table III). The decreased response at a low temperature is possibly due to the lower activity of the mosquitoes at this temperature. The probability of a decreased response with decreased activity has been pointed out by BAR-ZEEV (1960). The decreased response at the high temperature may be due to the difference in temperature on the dry and wet surface. At high temperatures the mosquitoes will probably avoid more strongly the higher of two temperatures as observed by THOMSON (1938) on *Culex fatigans.* It can be observed that at 35° C many more mosquitoes, per replicate, stood on the surface (N in table III) than they did at the other temperatures used, probably due to the cooler temperature on the wet surface. Another factor which might affect the response at different ambient temperatures is the resultant difference in saturation deficiencies. It has been found that the reaction of *Culex* (THOMSON, 1938) and *Tenebrio* (PIELOU & GUNN, 1940) are more in accord with relative humidity than with saturation deficiencies. With wireworms (LEES, 1943), on the contrary, the intensity of reaction is more in accord with saturation deficiencies.

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RESUME

RÉACTION DES MOUSTIQUES A LA MOITEUR ET A LA FORTE HUMIDITÉ

La description d'une méthode déterminant l'effet d'une surface moite et de l'air dans sa proximité (jusqu'à une distance de 2 cm) sur la femelle d'*Aedes aegypti* est présentée. Les insectes sont introduits dans une boîte de petri sous des conditions contrôlées de température et d'humidité ambiantes. Deux demi-ronds de papier filtre sont placés dans le fond de la boîte; l'un sec et l'autre moite. L'intensité de la réaction est déterminée par la moyenne du pourcentage de l'excédent enregistrant les positions respectives sur les côtés sec et moite.

Les moustiques femelles évitent fortement une surface moite, et modérément l'air dans sa proximité.

Les moustiques ne se nourrissent pas sur un bras humain préalablement humecté tant que la peau reste moite.

Les moustiques desséchés (perte d'eau 5.6% ou plus du poids du corps) sont fortement attirés par une surface moite, et modérément par l'air dans sa proximité; l'attraction augmentent avec la desiccation.

Les moustiques gravides évitent modérément une surface moite, mais ne réagissent pas à l'air dans sa proximité. Il existe des indications que les moustiques gravides doivent atteindre un „état de ponte", avant que la surface moite ne les attire.

L'évitement d'une surface moite et de l'air dans sa proximité augmente avec l'humidité relative ambiante.

L'évitement est plus faible à une température élevée de 35° C et basse de 18° C qu'à une température moyenne de 26° C.

Les causes des résultats contradictoires concernant les réactions des moustiques à la moiteur, obtenus par de nombreux investigateurs, sont discutées. Ils proviennent probablement des différences dans les techniques employées et dans l'état physiologique des moustiques.

L'évitement d'une surface moite est du type taxie, mais non du type cinèse.

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THE ECOLOGY OF THE FEMALE *HAEMATOPOTA INSIDIATRIX* AUSTEN (DIPTERA, TABANIDAE) IN SOUTHERN RHODESIA.

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The habitat of *H. insidiatrix,* in the region of Main Camp, Wankie National Park, is considered briefly. The seasonal occurrence of the fly is studied and its activity throughout the day. Comparisons are drawn with previous work in colder climates.

Present knowledge of the behaviour and ecology of tabanid flies is based very largely on studies in temperate or cold climates (CAMERON, 1926; TASHIRO & SCHWARDT, 1949; MILLER, 1951; DARLING, 1956 and KETTLE, 1957). Several authors, interested in the behaviour of large mammals, have observed the importance of blood-sucking female tabanids and recorded information on their biology (DARLING, 1956). Attempts to control and eliminate these insects have also been accompanied by ecological studies (MILLER, 1951). Only a few authors have been concerned primarily with understanding the ecological factors influencing tabanid behaviour in the field (in Europe: KETTLE, 1957; in N. America: TASHIRO & SCHWARDT, 1949; in Africa: HADDOW, GILLETT, MAHAFFY & HIGHTON, 1950 and HADDOW, 1952). HOCKING (1960) referred to the lack of knowledge concerning the population dynamics of tabanids and emphasised the need for such studies.

During visits to the Main Camp of Wankie National Park, Southern Rhodesia, in January and December 1959 and January 1960, the observations on the ecology of the female *Haematopota insidiatrix* Austen were undertaken which are recorded here. This study in a hot climate is compared with previous work in colder regions and an account is given of the habitat of the fly, its seasonal occurrence and its activity throughout the day. The position of the Main Camp is as follows: 18° 43′ S., 26° 57′ E., and 1073 m above mean sea level.

METHODS

Flies were collected off black cloth screens carried through the bush (BARRASS, 1959). Only the blood-sucking females settled and only these were studied. Activity was judged from the numbers settling on the screen. These were caught by placing a tube of 7.5 × 2.5 cm over them and later released approximately

100 yds. (90 m) behind the catching party. Measurements of air temperature and humidity were made using a whirling hygrometer held in the shade of the observer's body. The time of day is given in local time which is 2 hours in advance of G.M.T.

All experiments were carried out on the transects or the fly-round marked on the map (Figure 1). The term fly-round is well known in tsetse fly studies and is used here in the sense of Potts (1930) and Nash (1930).

RESULTS

The vegetation around Main Camp, Wankie National Park, is represented on the map (Figure 1) and described in more detail by Mitchell (in litt.). During January 1959 the fly-round was carried out 15 times. Stops were made every 100 yards (90 m) for the collection of flies. In Figure 2 the total number of flies collected at each stop is shown in relation to the vegetation types passed through on the fly-round. Many flies were caught in the *Acacia giraffe* bush and in the *Terminalia*, *Baekiaea* woodland. Few were caught in the bush formed of small trees

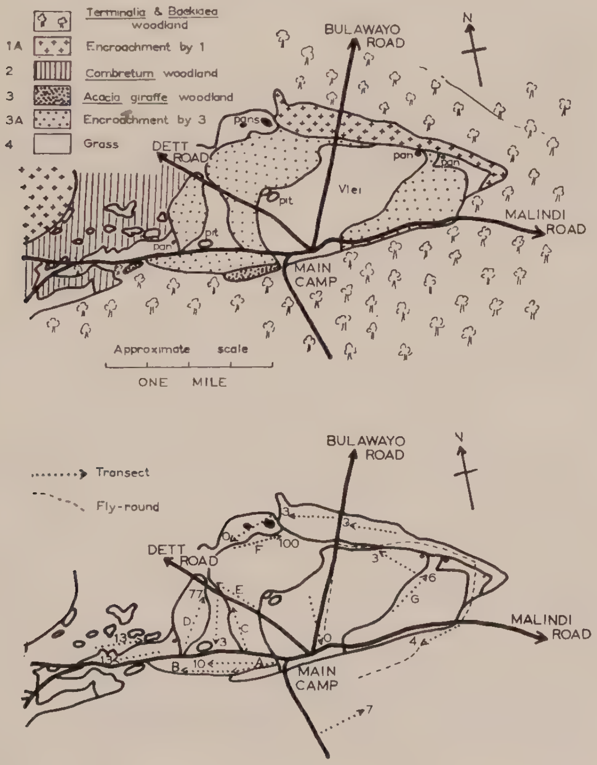

Figure 1. The vegetation around Main Camp, Wankie National Park, and the position of transects and a fly-round. Figures adjacent to transects are the number of flies caught. The letters indicate transects used in all-day catches.

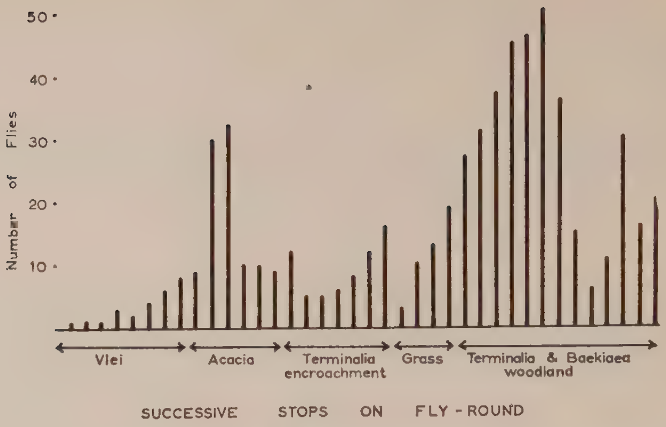

Figure 2. The total number of flies caught in the different vegetation types on the fly-round.

where encroachment of *Terminalia* and *Baekiaea* is taking place. Also, very few flies were caught when crossing grass and the majority of these were found where the grass bordered on to another vegetation type.

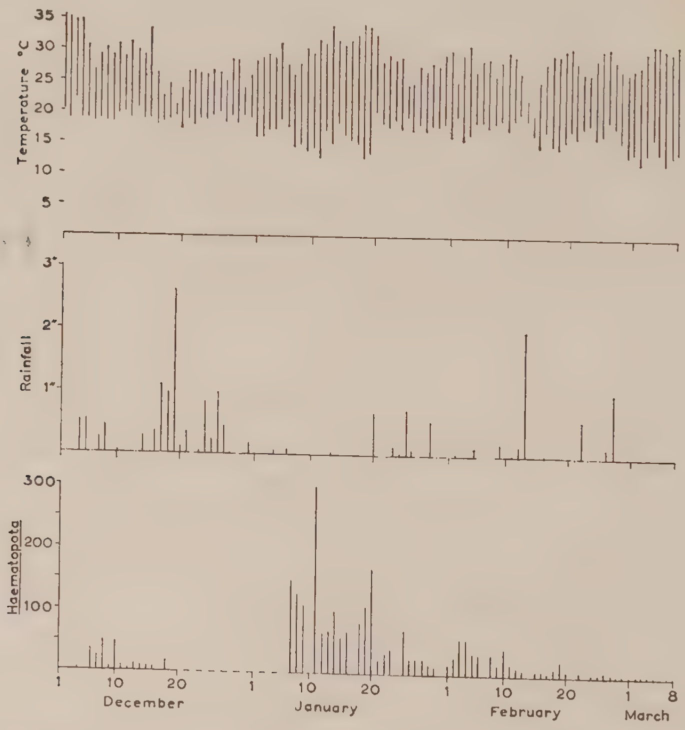

Figure 3. The maximum number of flies caught on days throughout the *Haematopota* season of 1959—60. The maximum and minimum temperatures and rainfall, registered at Main Camp, are also shown (records supplied by the Federal Meteorological Department).

On 14 Jan. 1960, the transects shown on the map were made with a stop every 45 m for the collection of flies. The number caught in each transect is also shown. These transects support the conclusion that flies may be caught in all habitats except open grassland. Large numbers of flies were caught in the *A. giraffe* bush (Transects D and F) but only a few elsewhere. Even within the *A. giraffe* great variations occur, in the number of flies caught. Some of the factors related to this variation are considered on the following pages.

During the rainy season of 1959 to 1960 the first specimens of *H. insidiatrix* were caught on 4 Dec. 1959, and the last on 8 Mar. 1960. In the course of experimental studies described in the present paper and elsewhere (BARRASS, 1960) the highest catch per 450 m transect was recorded each day (Figure 3). The record is incomplete in that no transects were made between 19 Dec. 1959 and 6 Jan. 1960.

Records of rainfall and daily temperature range are also shown in Figure 3. Rainfall is probably the most important environmental factor affecting tabanid emergence. Very little rain fell in early December. The catches during December were all lower than those for early January.

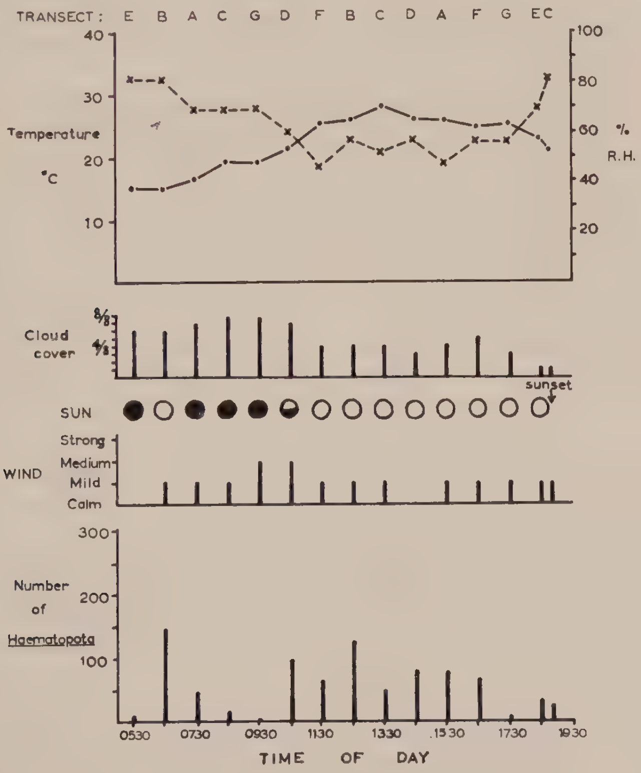

Figure 4. Number of *H. insidiatrix* caught on the transects shown for each hour of daylight on 7 Jan. 1960. Temperature, relative humidity, exposure of the sun, cloud cover and wind speed are also shown.

To determine the range of environmental conditions under which *H. insidiatrix* is active, 450 m transects were made through *A. giraffe* bush at intervals of 1 hr. from dawn until after sunset. The seven transects used in this study were all through the same type of vegetation and they are marked (A to G) on the map (Figure 1). All-day catches were made in this way on 3 days. The results are expressed in Figures 4, 5 and 6 so as to show the temperature and humidity at the start of each transect, cloud cover and whether or not the sun was obscured, wind (judged as being calm, mild, medium or strong), and the number of flies caught.

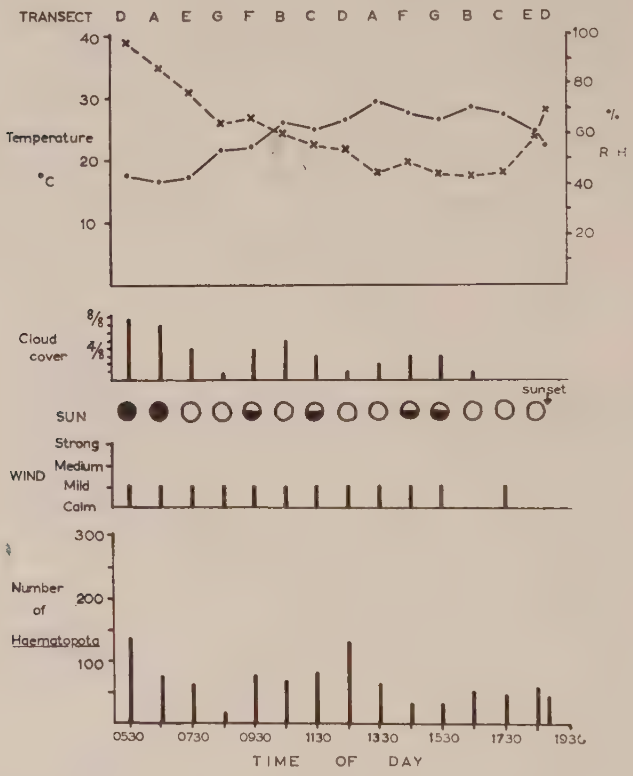

Figure 5. Number of *H. insidiatrix* caught on the transects shown for each hour of daylight on 8 Jan. 1960. Temperature, relative humidity, exposure of the sun, cloud cover and wind speed are also shown.

(a) *Day One*

7 Jan. 1960 (Figure 4) was cloudy, the sun was obscured early in the morning and the wind was mild for most of the day. The highest temperature recorded was 28.3° C and the lowest relative humidity was 46%. *H. insidiatrix* settled on the screens in considerable numbers at 06.30 hrs. and also between 10.30 and 16.30 hrs. Very low catches on transect G at 09.30 and 17.30 have probably reflect a smaller local population rather than temporarily adverse climatic conditions, since

low catches were also recorded on this transect on days two and three. Also fewer flies were caught on transects when the sun was obscured by cloud. Flies were caught immediately before sunrise and after sunset.

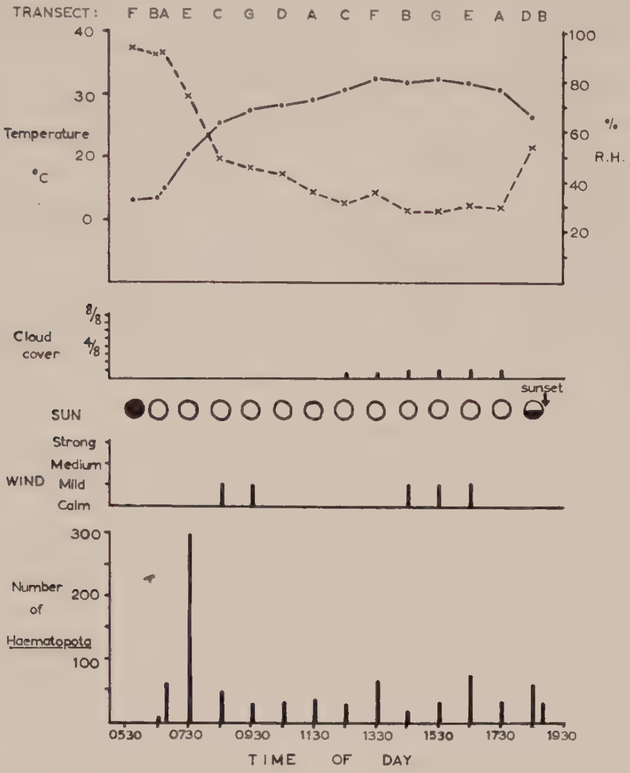

Figure 6. Number of *H. insidiatrix* caught on the transects shown for each hour of daylight on 11 Jan. 1960. Temperature, relative humidity, exposure of the sun, cloud cover and wind speed are also shown.

(b) *Day Two*

8 Jan. 1960 (Figure 5). Cloud obscured the sun early in the morning and intermittently throughout the day. The wind was mild or calm. The highest temperature recorded was again 28.3° C and the lowest relative humidity 43%. *H. insidiatrix* were caught before sunrise (light intensity = 4 lumens per square foot) and in considerable numbers throughout the daylight hours and immediately after sunset. The highest catches were both in transect D; approximately the same number of flies being caught at 05.30 and 12.30 hrs. The climatic conditions were opposite at these times as is clear from Figure 5.

(c) *Day Three*

11 Jan. 1960 (Figure 6). The sun was obscured by bush during the first transect; there was no cloud and very little wind for most of the day. The morning temperature was colder than on the two previous days and no flies were

caught on the first transect at 05.30 hrs. nor on the next transect at 06.30 hrs., except that 5 flies were caught at the last stop of this second transect (B). The temperature at his time was 14.7° C. Following this another transect (A) was started immediately and flies were caught at every stop (Total = 60). At 07.30 hrs. transect E was started and 298 flies were caught. The climate at this time was humid, warm, sunny and calm. This was the highest catch per 450 m on this day, and throughout the period of study. The day became very hot (max. = 32.8° C); the humidity was lower (min. = 28.5%) than on the two previous days and there was neither wind nor cloud. The catch of *H. insidiatrix* rarely exceeded 50. Flies were again caught after sunset.

The last transect of Day 3 was started at sunset and was continued for a further 500 m. In Figure 7 the light intensity and number of flies caught at each stop, from sunset onwards, are shown (it should be noted that the vertical scale in this figure is greater than in Figures 3, 4, 5 and 6). Flies were still being caught 40 minutes after sunset when they could hardly be seen on the screen and the only light was moonlight.

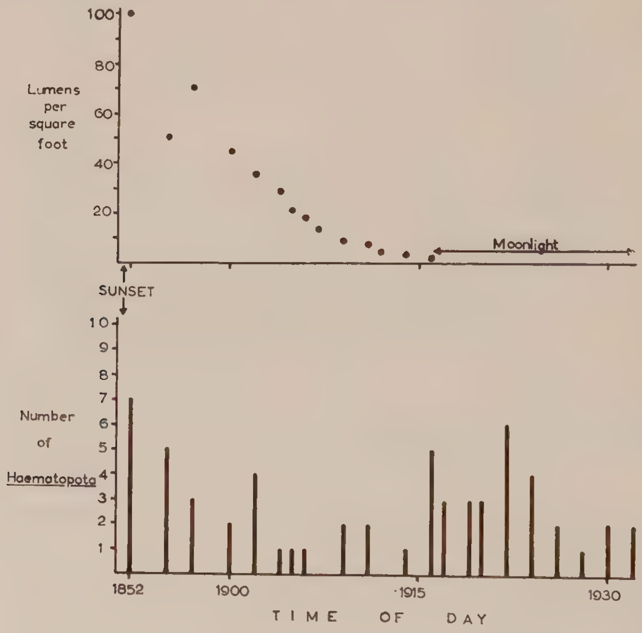

Figure 7. Showing the number of *H. insidiatrix* caught at successive stops at an increasing time after sunset. The light intensity for each time is also shown.

In January 1959 the fly-round (Figure 1) was completed five times a day on three separate fine and clear days. In Table I the number of flies caught at different times of day is shown. On each day more flies were caught in the morning than in the afternoon. On days 1 and 2 of the 14 hour catches no such difference was recorded but the temperature was never very high. Day 3 was cold at first and then very hot and a peak in numbers caught occurred at 07.30 hrs. It seems

TABLE I

The number of flies caught on the fly-round at different times of day on three separate days

Date	Flies caught between:				
	05.30 and 07.00	08.30 and 10.00	11.03 and 13.00	14.30 and 16.00	17.30 and 19.00
13 Jan. 1959	38	30	20	32	29
15 Jan. 1959	42	90	37	39	31
17 Jan. 1959	52	52	41	29	40
Totals	132	172	98	100	100

probable that these differences in activity throughout the day are related to temperature and humidity differences, rather than to other variables which occur irregularly.

DISCUSSION

In temperate climates tabanids are active on warm, sunny days (IMMS, 1957). KETTLE (1957) recorded the biting activity of *Haematopota pluvialis* L. over a 24 hour period in Scotland. During the hot bright weather of the first day this insect was active until sunset but on the following morning, which was cold and dull, it was inactive. DARLING (1956), also in Scotland, found *H. pluvialis* to be most active in bright sunlight in hot, still weather. He also states that biting occurs over a wide range of atmospheric conditions. OLSUF'EV (1935) says that *Haematopota* sp. does feed at temperatures below 16° C and also in dull weather and light rain.

In tropical Africa, HADDOW *et al.* (1950) and HADDOW (1952) found that *Chrysops centurionis* Austen is most active immediately after sunset and that *Haematopota nefanda* Edw. has a biting cycle with peaks in the early morning and evening.

In the present study *H. insidiatrix* females settled on the screen over a wide range of climatic conditions. The lowest temperature at which flies were caught was 14.7° C. The highest catch was made in the early morning in calm, humid, hot and sunny weather. Very high temperatures, low humidity and a low catch of flies seem to go together and the results shown in Table I suggest a morning peak of activity on a hot, sunny day. Cloud obscuring the sun or a gust of wind are immediately followed by a reduced catch; though flies were caught even when cloud covered the whole sky or when a mild wind was blowing continuously. No work was done on the effect of rainfall on activity but *H. insidiatrix* were observed flying around a motor vehicle during a heavy rainstorm.

So far as is known, the Tabanidae of Nigeria are diurnal (CROSSKEY & CROSSKEY, 1955). BEQUAERT (quoted by HADDOW, 1952) considered that nocturnal habits are probably far more common in the Tabanidae than is realised, particularly in the tropics. In the present study *H. insidiatrix* were caught 40 minutes after sunset on a moonlit night but no information is available concerning activity on a moonless night.

Haematopota spp. seem to be most active under similar conditions in both temperature and tropical climates. DARLING (1956) found *H. pluvialis* most active in hot sunny weather in Scotland and he records the temperature at this time as 33.9° C in the shade. He does not record humidity. On the other hand very high temperatures and low humidities were associated with reduced catches in the present work. In N. Nigeria *H. pertinens* is more prevalent on dull, overcast days than on days when the sun shines unobscured (CROSSKEY & CROSSKEY, 1955). In Nyasaland, NEAVE (1912) says that the female *Haematopota* usually bites in the rather dull damp weather and rarely in the heat of the sun. In very sunny weather they are most in evidence from about 08.00 to 10.00 hrs. and again from 16.00 hrs. to sunset.

Differences in the activity of *Haematopota* on hot sunny days in temperate and tropical climates may be explained in terms of temperature and humidity differences. Laboratory studies under controlled conditions are needed to determine whether or not these differences in activity are also related to differences between species.

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ZUSAMMENFASSUNG

DIE ÖKOLOGIE DER WEIBLICHEN HAEMATOPOTA INSIDIATRIX *AUSTEN (DIPTERA, TABANIDAE) IN SÜD-RHODESIEN*

Die Heimat von *H. insidiatrix*, in der Nähe von Main Camp, Wankie National Park, wird kurz betrachtet. Das jahreszeitliche Vorkommen der Fliege und ihre Aktivität im Tagesverlauf werden untersucht. Die Resultate werden mit denen von früheren Untersuchungen in kälteren Klimaten verglichen.

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CHILL-COMA AND COLD DEATH TEMPERATURES OF *APIS MELLIFERA*

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Worker bees have a lower chill-coma temperature than drones or queens, which is influenced by their acclimatisation temperature as is their food consumption at a given temperature. Most bees die after 50 hrs. in chill coma, and survive longer at 5° C than at 0 or 10° C. Cold death occurs between —2 and —6° C and is unaffected by acclimatisation. Winter bees have lower chill-coma temperatures than summer bees but after acclimatisation to 35° C there is no difference. The chill-coma temperature of summer bees decreases with age irrespective of acclimatisation.

The present investigation is complementary to a study of the relationship between temperature and metabolism in honeybees (FREE & SPENCER-BOOTH 1958 & 1959). The chill-coma temperature is the highest temperature at which bees are immobilised by cold and the cold-death temperature the highest at which bees are killed by cold.

GENERAL METHOD

A method similar to that of MELLANBY (1939) was used. Single bees in cages with sugar-syrup and water were acclimatised at 20 or 35° C for 24 hrs. They were then transferred to test tubes which were placed in vacuum jars containing water cooled by crushed ice, and salt when necessary, to the experimental temperature. After one hour the tubes were removed and records made as to whether the bees were (a) moving, (b) motionless but recovered later (in chill coma), (c) or dead, i.e. motionless and did not recover.

RESULTS AND DISCUSSION

Chill-coma temperatures of workers, drones and queens

Workers, drones and queens were kept for 24 hours at 35° C (each queen was accompanied by 2 workers) before they were transferred to temperatures of 9—15° C. The chill-coma temperature varied with individuals of the same caste but in general was lower for workers than for drones or queens (Table I).

KALABUKHOV (1934) found that drones and queens died sooner than workers at low temperatures. Drones and queens fly only at temperatures much above the minimum at which workers fly.

TABLE I

Chill-coma temperatures of workers, drones, and queens

Environmental temperature (° C)	Workers		Drones		Queens	
	No. moving	No. still	No. moving	No. still	No. moving	No. still
9	2	6	0	6	—	—
10	3	7	0	11	1	4
11	20	13	3	15	—	—
12	19	4	2	25	5	11
13	11	0	8	23	—	—
14	32	0	46	19	6	0
15	19	0	25	4	—	—

Effect of acclimatisation on chill-coma temperature

Workers were acclimatised for 24 or 48 hrs. at 20 or 35° C (Table II). Equal numbers of each category were usually tested together at the same temperature. Significantly more bees acclimatised for 24 hrs. at 35° C than at 20° C became comatose at 10 and 11° C. ($P < 0.001$, $P < 0.01$ respectively). Acclimatisation for longer than 24 hrs. had little or no further effect.

TABLE II

Acclimatisation and chill coma (Worker bees)

Period of acclimatisation	24 hrs.				48 hrs.			
Temp. of acclimatisation (° C)	20° C		35° C		20° C		35° C	
Environmental temperature (° C)	No. bees moving	still	No. bees moving	still	No. bees moving	still	No. bees moving	still
7	0	2	0	2	—	—	—	—
8	0	4	0	4	0	2	0	2
9	4	6	0	10	1	3	0	4
10	13	2	1	15	6	1	0	10
11	15	1	7	9	3	0	2	2
12	21	0	20	0	—	—	4	0
13	2	0	2	0	—	—	—	—

In another experiment, bees were kept singly at (a) 20° C for 24 hrs. (b) 35° C for 24 hrs. (c) 20° C for 18 hrs. and 35° for 6 hrs. The numbers of moving to still bees after one hour at 9° C were, 17 : 16, 5 : 44, and 10 : 28 respectively, indicating that category 'c' bees were only partially acclimatised to 35° C.

Finally, groups of 100 bees were kept for 24 hrs. at 35° C and 20° C. The temperatures maintained in the groups were about 35° C and 28° C respectively (FREE & SPENCER-BOOTH 1958). Significantly more ($P < 0.01$) of the 35° C than of the 20° C bees were comatose when put singly at 10° C (Table III).

The above results agree with those of MELLANBY (1939, 1958) on other insects, although individual honeybees vary more, possibly because their resting

TABLE III

Effect of acclimatising groups of 100 bees to different temperatures on chill coma

Temperature of acclimatisation	20° C		35° C	
Environmental temperature (°C)	No. bees moving	 still	No. bees moving	 still
9	3	18	0	27
10	20	15	6	21
11	9	2	7	3

metabolism increases at low temperatures (FREE & SPENCER-BOOTH 1958, 1959), or because they are more restless than non-social insects when isolated at low temperatures.

Effects of acclimatisation on metabolic rate

To determine the effect of previous acclimatisation on food consumption and hence metabolic rate at a given temperature, the following experiment was done 5 times. 4 Groups, of 25 workes bees each, were put in cages and given sugar syrup. Groups A & B were put at 20° C and 35° C respectively for 2 days; group C was at 20° C on day 1 and at 35° C on day 2; group D was at 35° C on day 1 and 20° C on day 2. (Table IV). Bees kept at the same temperature throughout the experiment (Groups A & B) took less food on day 2 than day 1. Acclimatisation at 20° C increased subsequent food consumption at 35° C ($P < 0.01$) but acclimatisation at 35° C did not reduce subsequent food consumption at 20° C which was probably at the minimum level for that temperature.

TABLE IV

Effect of acclimatisation temperature on food consumption

		Group A	 B	 C	 D
Temp. (°C)	Day 1	20	35	20	35
	„ 2	20	35	35	20
Mean amount of food (mm³) taken per bee.	Day 1	71.2 ± 4.1	46.0 ± 2.8	74.8 ± 4.6	45.7 ± 4.3
	„ 2	44.4 ± 2.8	23.8 ± 3.1	40.6 ± 2.1	44.5 ± 2.6

GUNN & HOPF (1942) found the acclimatisation temperature affected the rate of activity of *Ptinus tectus* but MELLANBY (1939) found it had no effect on the rate of activity of *Cimex lectularius*.

Recovery from chill coma

Bees were acclimatised singly to 20 or 35° C for 24 hrs. and then kept at 5° C for 1 or 5 hrs. The tubes containing them were then removed from the vacuum jars and the bees observed *in situ* at room temperature (16—20° C), one bee from each acclimatisation temperature being watched simultaneously. The mean time taken before the first movement (usually twitching an antenna or leg) was less for bees acclimatised at 20° C than at 35° C, and less for bees in chill coma for 1 hr. than for 5 hrs. (Table V). In all of 14 comparisons bees acclimatised at 20° C moved before those acclimatised at 35° C when kept for 1 hr. at 5° C and in 11 out of 14 comparisons when kept for 5 hrs. at 5° C. These results agree with those of Mellanby (1939) on other insects.

TABLE V

Mean time (sec) for recovery of bees from chill coma (14 bees in each category)

Length of time (hrs) in chill coma	1		5	
Acclimatisation temp. (° C)	20	35	20	35
Mean time (sec) before first movement	143.3 ± 13.6	323.9 ± 33.5	325.2 ± 38.7	487.8 ± 47.1

Length of life in chill coma

Bees acclimatised at 35° C were kept singly at 0, 5 or 10° C for different periods. They were then transferred to 35° C and those that moved kept with food for a further 24 hrs. to find if their recovery was complete (Table VI). Few bees survived being chilled more than 80 hrs. and most no more than 50 hrs. In general, they survived better at 5° C than at the other temperatures. Perhaps this was because at 0° C use of blood sugar is disturbed and assimilation of gut contents ceases, and at 10° C sugar in the gut and blood is more rapidly metabolised than at 5° C. (See Parhon 1909, Kosmin, Alpatov & Resnitschenko 1932, Kalabukhov 1933 & 1934).

Although bees consume little food during short periods in chill coma, the following experiment indicates that food consumption probably increases during cooling and recovery. 100 bees were kept in a cage with food at 35° C. The food was removed and 10 of the bees put singly at 5° C for 1 hr; they were then transferred to 35° C to recover and 1 hr. later their honey-stomachs and contents weighed, together with those of 10 bees taken directly from the cage. On each of the 6 times this experiment was done the honey-stomachs of bees that had been in chill coma weighed less than those of the control bees (means of 13.1 and 18.9 mg. respectively for all experiments). These results agree with those of Free & Spencer-Booth (1959) on the increased food consumption of small groups of bees at low temperatures.

Cold-death temperatures

Bees were subjected to temperatures below 0° C for ½, 1 or 3 hrs. Every effort was made to keep the bees dry and so prevent frozen water on their cuticles inoculating their body fluids (Salt 1958). Those that moved on warming to room temperature were kept for 24 hrs. to find if their recovery was temporary or permanent. Some bees died at —2° C and nearly all at —6° C (Table VII). Exposing bees for ½ hr. or 1 hr. gave similar results but the death rate between —3 and —5° C was greater after 3 hrs. exposure, indicating that death resulted from some cumulative process at these temperatures. Previous acclimatisation to 20 or 35° C had no differential effect; this is contrary to the results of Mellanby (1939) with *Blatta orientalis.*

TABLE VI *Survival of bees after exposure to cold*

Duration of exposure (hrs)	Temperature of exposure (°C) 0 No. bees died	 0 No. bees recovered temporarily	 0 No. bees recovered completely	 5 No. bees died	 5 No. bees recovered temporarily	 5 No. bees recovered completely	 10 No. bees died	 10 No. bees recovered temporarily	 10 No. bees recovered completely
5	0	0	2	0	0	1	0	0	5
20	—	—	—	0	0	1	0	0	1
30	1	2	13	0	0	6	0	3	20
40	4	3	23	—	—	—	—	—	—
50	20	5	6	12	0	23	14	4	11
60	14	3	9	16	1	11	14	1	8
70	15	0	0	18	3	11	17	1	2
80	39	2	2	16	2	9	24	0	7
100	10	0	0	9	0	0	14	2	3

TABLE VII *Cold-death temperatures*

Environmental temperature (° C)	Duration of exposure ½ hr No. bees died	 ½ hr No. bees recovered temporarily	 ½ hr No. bees recovered completely	 1 hr No. bees died	 1 hr No. bees recovered temporarily	 1 hr No. bees recovered completely	 3 hrs No. bees died	 3 hrs No. bees recovered temporarily	 3 hrs No. bees recovered completely
Bees acclimatised 24 hrs at 20° C									
— 2	0	0	5	0	1	2	1	1	3
— 3	0	2	4	3	3	9	3	6	1
— 4	9	2	4	16	8	10	11	3	1
— 5	16	1	3	14	2	9	5	2	0
— 6	6	0	0	23	0	0	1	0	0
— 7	3	0	0	11	0	0	—	—	—
— 8 to — 10	6	0	0	6	0	0	—	—	—
Bees acclimatised 24 hrs at 35° C									
— 2	0	1	5	0	0	3	1	2	3
— 3	0	0	6	1	5	7	5	5	1
— 4	6	4	8	13	10	4	11	3	1
— 5	15	0	6	8	7	5	7	2	0

Effect of age and occupation on chill coma

At intervals from June to Sept., during 2 years, newly emerged bees were marked and introduced to colonies. They were removed at various ages and subjected to low temperatures either directly or after acclimatisation at 35° C for 24 hrs. (Table VIII, next pages).

The results for bees up to 7 days old were similar, whether acclimatised at 35° C or not; this was to be expected because bees of this age tend to keep to the brood nest (FREE 1961) where the temperature is about 35° C in summer (see RIBBANDS 1953). The chill-coma temperature decreased greatly with age for bees not previously acclimatised at 35° C. This was probably because bees which have become foragers tend to keep away from the brood nest when inside the hive and so are subject to lower temperatures than younger bees, particularly at night. Perhaps this results in their remaining more active and flying more readily at low temperatures, as suggested by the results of ALLEN (1959).

Even after acclimatisation at 35° C, older bees had a lower chill-coma temperature than younger bees although the difference was less, so a factor other than acclimatisation must also be involved. When bees are anaesthetised with CO_2 they forage and die earlier than normal (RIBBANDS 1950); however, we found that anaesthetising bees with CO_2 does not affect their chill-coma temperature. Younger bees have larger fat bodies than older bees (SIMPSON 1956) and we thought that a bee's chill-coma temperature might be related to its protein and fat reserves. To test this, newly emerged bees which need protein for their fat bodies and hypopharyngeal glands to develop (LOTMAR 1939; MAURIZIO 1950) were kept in groups of 100 at 35° C for one week and fed either protein and carbohydrate or carbohydrate only (Table IX). The bees fed protein had a lower chill-coma temperature than the protein-starved bees ($P < 0.02$ at 11° C) indicating that the chill-coma temperatures of younger bees was not higher because of their higher protein content.

TABLE IX

Effect of protein feeding on chill coma

	Bees fed protein and carbohydrate		Bees fed carbohydrate only	
Environmental temperature (° C)	No. moving	No. still	No. moving	No. still
9	0	9	0	7
10	23	37	12	47
11	23	23	8	36
12	27	9	24	12
13	2	1	3	0

However, young bees have a lower metabolic rate than older bees (ALLEN 1959) and bees with different metabolic rates have different chill-coma temperatures, e.g. at 35° C bees have a lower metabolic rate and a higher chill-coma temperature than at 20° C (see above). Therefore the difference in chill-coma temperature between bees of different ages is probably related to their metabolic rates.

An obvious biological value to honeybees of acclimatisation to low temperatures

TABLE VIII

Chill-coma temperatures of bees of known ages

	Bees taken directly from hive						
Age of bees (days)	1—7		8—14		15—21		22—2
Environmental temperature (° C)	No. moving	No. still	No. moving	No. still	No. moving	No. still	No. moving
8	—	—	0	1	—	—	—
9	0	5	0	5	0	8	1
10	1	21	11	17	11	16	15
11	7	35	15	22	32	33	23
12	12	17	20	3	24	3	17
13	4	1	4	0	5	0	5
14	—	—	2	0	—	—	1

would be an increased ability to survive cold during winter. A summary of the results for bees tested during summer and winter (Table X) indicates that, although bees direct from the hive had a lower chill-coma temperature in winter than summer, after being acclimatised to 35° C this difference disappeared. On 17 days bees were collected from the centre and edge of winter clusters and subjected to low temperatures (Table XI). The temperature in the centre of a winter cluster remains at 20—30° C (GATES 1914, WILSON & MILUM 1927, CORKINS 1930) so bees in different parts of it would be acclimatised (partially or completely) to different temperatures. This is reflected in the results.

TABLE X

Comparison of chill-coma temperature of summer and winter bees

	Bees taken directly from hive				Bees acclimatised 24 hrs at 35° C			
	Summer		Winter		Summer		Winter	
Environmental temperature (° C)	No. moving	No. still	No. moving	No. still	No. moving	No. still	No. moving	No. still
7	—	—	—	—	—	—	0	2
8	0	16	0	32	0	18	0	24
9	4	64	76	85	10	62	5	58
10	103	108	94	27	45	141	3	49
11	150	116	35	4	135	198	10	15
12	115	28	5	1	131	51	24	0
13	54	2	—	—	46	11	2	0
14	6	0	—	—	34	0	—	—
15	—	—	—	—	19	0	—	—

We thank Miss Eileen Macrow for her assistance during the experiments and Dr. K. Mellanby and our colleagues of the Bee Department for criticising the manuscript.

E VIII (continued)

	Bees acclimatised at 35° C for 24 hrs									
	1—7		8—14		15—21		22—28		29 +	
No. still	No. moving	No. still	No. moving	No. still	No. moving	No. still	No. moving	No. still	No. moving	No. still
3	0	4	0	10	0	3	—	—	0	1
24	2	13	1	15	2	10	2	6	1	12
33	3	25	7	36	7	13	6	10	18	49
10	7	38	12	57	16	20	7	8	66	62
2	15	12	18	23	7	4	13	4	54	5
1	8	4	15	2	1	1	2	1	7	0
—	—	—	—	—	—	—	1	0	1	0

TABLE XI

Chill-coma temperatures of bees from edge and centre of winter cluster

	Bees from edge		Bees from centre	
Environmental temperature (° C)	No. moving	No. still	No. moving	No. still
8	0	12	0	20
9	48	30	28	55
10	54	11	40	16
11	20	0	15	4
12	3	0	2	1

ZUSAMMENFASSUNG

KÄLTESTARRE- UND KÄLTETOD-TEMPERATUREN VON APIS MELLIFICA

Arbeitsbienen haben eine niedrigere Kältestarretemperatur als Drohnen und Königinnen. Akklimatisierung der Arbeiterinnen an verschiedene Temperaturen beeinflußt ihre Kältestarretemperaturen und die Zeit, die sie zur Erholung von der Kältestarre benötigen. Die Akklimatisation ist nach 24 Stunden vollständig. Die Stoffwechselrate der Bienen bei einer gegebenen Temperatur wird ebenfalls von der Akklimatisationstemperatur beeinflußt.

Die meisten Bienen sterben nach 50stündiger Kältestarre und wenige überleben mehr als 80 Stunden. Bei 5° C ist die Überlebensrate größer als bei 0° oder 10°. Der Tod tritt zwischen —2° und —6° C ein und ist von vorangegangener Akklimatisation unbeeinflußt.

Bienen vom Rande einer Wintertraube haben niedrigere Kältestarretemperaturen als Bienen aus der Mitte. Winterbienen haben eine niedrigere Kältestarretemperatur als Sommerbienen, doch nach Akklimatisation an 35° C besteht kein Unterschied mehr. Die Kältestarretemperatur nimmt im Sommer mit dem Alter ab, ob die Bienen an 35° C akklimatisiert sind oder nicht. Es wird vermutet, daß dies auf Unterschieden in der Stoffwechselrate der betreffenden Bienen beruht.

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THE FOOD REQUIREMENTS BY WEIGHT OF SOME BRITISH NABIDAE (*HETEROPTERA*)

BY

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Experiments in which the weight of prey eaten was determined for each nymphal instar of *Stalia major* Costa, gave an overall conversion ratio of live weight increase to wet weight of food eaten of 0.51. The conversion ratio did not vary with temperature in the range 13.5 to 22° C. A simple relationship between the initial weights of successive instars, i.e. that they follow a regular geometric progression, is shown for *S. major* and is used to calculate mean initial weights for nymphs of 3 other species of Nabidae. The results are confirmed by comparison with weights of field captured specimens.

The Nabidae are a family of terrestrial Heteroptera, all members of which are general predators. Their prey consists of other insects (from at least nine Orders), spiders and mites (FEWKES, 1958). There are thirteen British species; the immature stages are described by SOUTHWOOD & FEWKES (in press), the adults by SOUTHWOOD & LESTON (1959).

The purpose of this work was to obtain information contributing towards a measurement of the value of the Nabidae as predators that can be applied to naturally occurring populations. Feeding experiments were conducted to determine the weight of prey eaten by each instar of one species, *Stalia major* Costa, and the consequent weight increases. From the results a conversion ratio of live weight increase to wet weight of food eaten can be determined which, if applicable to nabids generally, may be used to calculate the weight of food eaten by each instar of each species for which the mean initial weight of each instar is known.

MATERIALS AND METHODS

S. major, a fairly common species in grassland, was reared from eggs found by splitting old *Dactylis glomerata* L. stems. In the laboratory, all stages fed readily on small caterpillars, various leafhoppers and aphids. The predator usually captures its prey by pouncing on it and holding it down with its forelegs. The proboscis is inserted either at the moment of seizure or immediately afterwards; the prey is not eaten entirely, but is sucked, the exoskeleton and varying amounts of internal tissue being discarded.

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For the experiments a prey species was required that was continuously available in large numbers, easy to handle, and acceptable to the predators. These requirements were met by using the larvae of *Carpophilus dimidiatus* Fab. (Coleoptera, Nitidulidae). This stored products pest was cultured on rolled oats and yeast.

Predators and prey were kept in two by one inch flat bottomed specimen tubes stoppered with muslined perforated corks. These cages were enclosed in fairly airtight plastic boxes with water to a depth of approximately half an inch, so that the humidity was kept high continuously, at all temperatures. Experiments were carried out at two temperatures; in the laboratory at 13.5 to 19° C (mean 15.5° C), and in a controlled temperature room at 20 to 22° C (mean 21° C).

Estimates of the weight of food eaten by the predators were obtained by feeding them on weighed last stadium larvae of *C. dimidiatus*, and re-weighing the sucked prey within 24 hours. The nymphs were kept singly and one prey was made available each day. The nymphs were weighed on hatching and after each moult, when the exoskeleton had hardened. A torsion balance was used for the weighings, except of the heavier instars and adults for which a beam balance was used.

In order that the results obtained under laboratory conditions might be checked against what actually happens in the field, some weighings were made of field captured specimens.

RESULTS

Relationship of weight eaten to weight increase

The values obtained for the weights eaten and weight increases in the experiment at 13.5 to 19° C were simular to those obtained at 20 to 22° C. Temperature affects the rate of feeding (the predators kept at the higher temperature developed more quickly), but not the actual weight eaten or the conversion ratio (Table I). The fact that the conversion ratio does not vary with temperature between 13.5 and 22° C means that the results can probably be used at fluctuating temperatures in that range.

TABLE I

Stålia major. *Comparison of the results of feeding experiments at 15.5° C (given first) and 21° C (given below the 15.5° C results).*

Instar	Duration (days)	Weight consumed (milligrams)	Weight increase (milligrams)
I	11.3	0.81 (0.70—0.99)	0.36 (0.29—0.46)
	7.4	0.90 (0.41—1.46)	0.38 (0.13—0.49)
II	10.1	1.68 (0.71—2.33)	0.68 (0.48—0.89)
	7.5	1.60 (1.04—1.93)	0.65 (0.55—0.79)
III	12.0	2.99 (2.45—3.33)	1.41 (0.86—2.07)
	9.1	2.67 (2.07—3.16)	1.44 (1.23—1.69)
IV	12.6	7.71 (7.58—7.84)	3.91 (3.80—4.02)
	9.0	5.27 (4.75—5.59)	2.78 (1.91—3.45)
V	17.8	—	—
	—	11.67 (one nymph)	6.25

The results are pooled in Table II, which gives the mean values of the initial weight, weight increase, weight consumed and conversion ratio for each instar. Discrepancies between the first column (initial weight) and the second (initial weight of next instar) are due to the fact that only successful nymphs could be considered in assessing the conversion ratios. Lighter first and second instars were, on the average, less successful than heavier ones.

TABLE II

Stålia major. *Pooled results of all experiments (all weights in milligrams)*

Instar	Initial weight	Initial weight of next instar	Weight increase	Weight consumed	Conversion ratio
I	0.31	0.69	0.38	0.88	0.43
II	0.74	1.42	0.68	1.66	0.41
III	1.54	2.97	1.43	2.83	0.51
IV	2.98	6.21	3.23	6.25	0.52
V	6.00	12.25	6.25	11.67	0.54
I to V	0.31	12.25	11.94	23.29	0.51

The conversion ratios for first and second instars are lower than those for later instars. This may be due to a real difference in physiological efficiency. However a simpler explanation is that, as the predators were all kept in similar sized cages, the ratio of searching area to size of predator was higher for earlier than for later instars so that the energy needed to obtain each meal was proportionately greater, more food per unit increase in weight being needed for earlier instars. The overall conversion ratio of 0.51 is probably more characteristic of the species than the individual ratios for each instar.

A few first instar nymphs of two other nabids, *Nabis flavomarginatus* Scholtz and *Dolichonabis limbatus* Dahlbom, kept under similar conditions to those described for *S. major*, were found to have mean conversion ratios of 0.40 and 0.49 respectively.

There does not seem to have been much work on determining such conversion ratios for insects. BUXTON's (1930) figures may be used to calculate comparable ratios for *Rhodnius prolixus* which feeds on mammalian blood. These vary from 0.32 for the first instar nymphs to 0.20 for fifth instars, with an overall conversion ratio of 0.22. It is interesting to note that the earlier instars here appear more efficient than the later ones in converting their food to body tissue. A more striking difference to the entomophagous Nabidae is the comparitively low overall conversion ratio. *S. major* is more than twice as efficient as *R. prolixus* in converting its food to body tissue.

Phytophagous insects also seem to be less efficient in converting their food to insect tissue: EVANS (1939 a, b) gives figures for the weight of food consumed and the live weight increase of *Phalera bucephala* L. (Lepidoptera, Notodontidae) and *Aglais urticae* L. (Lepidoptera, Nymphalidae). The conversion ratios are 0.34 and 0.26 respectively. Other authors deal only with dry weights. CROWELL (1941) gives a dry weight increase to dry weight eaten ratio of 0.34 for *Prodenia eridania* Cram. (Lepidoptera, Noctuidae), and quotes figures of 0.31 to 0.32 for *Blattella germanica* L. (Dictyoptera, Blattidae), and 0.16 for *Aglais urticae.*

It seems to be of considerable advantage for an insect to feed on insect tissue rather than on mammalian blood or on vegetation. A higher proportion of the food is useable, and there is consequently less waste to be excreted.

Weight increase from instar to instar

When weight is plotted against time, an S-shaped curve results. A straight line relationship is obtained by using a one third power transformation for the weights; this is equivalent to converting weight to a linear dimension (cf. RICHARDS, 1949). However a somewhat better fit for the data on *S. major* is found by postulating that the initial weights of successive instars follow a regular geometric progression. This relationship, which does not depend on knowing the duration of each instar, may be expressed thus:

$$W_n = W_a . K^{n-a}$$

Where; W_a, W_n = initial weights of nth, ath instars;
K = the constant factor of weight increase.

K may be calculated from the initial weights of any two instars or, more accurately from a series of initial weights. For example, using the weights obtained for *S. major;*

$W_1 = 0.31$ milligrams,
$W_5 = 6.21$ milligrams,

therefore $K^4 = \frac{6.21}{0.31}$

i.e., $K = 2.115$.

Expected values for the initial weights of the intermediate instars, (2nd to 4th), may be calculated from the initial weight of the first instar and the value obtained for K for example:

$W_3 = W_1 . K^2$
$= 1.39$.

These calculated values are compared in Table III with the actual values ob-

TABLE III

Stålia major. *Comparison of initial weights of laboratory reared specimens, calculated initial weights (using a factor of regular geometric increase = 2.115), and weights (within instar) of field captured specimens (all weights in milligrams)*

Instar	Mean initial weight of laboratory reared specimens	Calculated initial weight	Weights of field captured specimens (within instar weights)
I	0.31 (0.28 to 0.34)	0.31	—
II	0.69 (0.46 to 0.80)	0.66	—
III	1.42 (1.26 to 1.66)	1.39	3.13, 3.28
IV	2.97 (2.45 to 3.73)	2.93	3.76 to 6.00
V	6.21 (4.80 to 7.75)	6.20	7.00 to 12.00
Adult	12.25 (one male)	13.12	8.75 to 13.00 (males) 12.00 to 21.00 (females)

tained in the feeding experiments and with a range of values obtained by weighing field captures. The close agreement between the first two columns demonstrates the excellent fit for the hypothesis that there is a regular geometric increase in initial weights of successive instars. The weights of field captures for any one

TABLE IV

Comparison of expected initial weights, calculated by using a factor of regular geometric increase (K), (calculated from the hatching weight and the initial weight of another instar estimated by weighing field captured specimens which moulted soon after capture), and weights of field captured specimens of three species of Nabidae (all weights in milligrams)*

N. rugosus (K = 2.224)	Instar	Calculated initial weight	Weights of field captured specimens
	I	0.12	0.11 to 0.13 (initial weights)†
			0.13 to 0.28 (all weights)
	II	0.27	—
			0.53 to 0.74
	III	0.59	0.55
			0.55 to 1.68
	IV	1.32*	1.16 to 1.83
			1.16 to 4.19
	V	2.94	3.05
			2.73 to 8.00
	Adult	6.53	—
			4.50 to 7.75 (males)
			5.50 to 8.00 (females)
N. flavomarginatus (K = 2.155)			
	I	0.31	0.30 to 0.33
			0.19 to 0.70
	II	0.67	0.45, 0.51
			0.41 to 1.48
	III	1.44*	1.32 to 1.54
			1.17 to 3.37
	IV	3.10	2.60
			1.93 to 8.00
	V	6.69	5.90
			5.00 to 16.00
	Adult	14.41	—
			10.50 to 17.00 (males)
			10.50 to 28.00 (females)
D. limbatus (K = 2.214)			
	I	0.31	0.29 to 0.35
			0.27 to 0.69
	II	0.69	0.57, 0.59
			0.57 to 1.66
	III	1.52*	1.26 to 2.78
			1.26 to 3.62
	IV	3.36	3.04 to 4.02
			3.04 to 4.98
	Adult	7.45	—
			4.99 to 8.50 (males)
			8.00 to 19.00 (females)

†) (initial weights) and (all weights) given as indicated for *N. rugosus* I instar

instar fall in the main between the mean initial weight of that instar and the mean initial weight of the successive instar as determined for the laboratory reared specimens. The high weights recorded for field captures of third instars are explained by the fact that the two specimens weighed were fully fed and soon after capture moulted to the fourth instar. First and second instar nymphs were never taken in the field, but the initial weight of the first instar may be assumed to be the same for eggs hatching in the field as for those hatching in the laboratory, as the latter were collected from the field. There was no difference between the weights of first instar nymphs hatching from eggs kept at 13 to 19° C and at 20 to 22° C.

Applying the same principle to other Nabidae (*Nabis rugosus* L., *N. flavomarginatus* Scholtz, and *Dolichonabis limbatus* Dahlbom), good agreement between calculated weights and the weights of field captures is obtained. (Table IV). K is slightly different for each species; it is obtained from the hatching weight and the mean initial weight of an intermediate instar, obtained by weighing several field captures which moulted within 24 hours of captivity.

Application of results

An estimate may be made for each instar of each of the species mentioned above of the mean weight of prey eaten per day at a given temperature. This assumes that the conversion ratio of 0.51 for *S. major* is applicable to the other species, also that the duration of each instar at a given temperature is known. The estimate is termed the predatory value of the nymph concerned.

This work was done in the Department of Zoology and Applied Entomology at the Field Station of Imperial College, Sunninghill, Ascot, Berkshire. I wish to thank Professor O. W. Richards for accomodation and facilities. I also wish to thank Professor Richards, and Dr. T. R. E. Southwood for their guidance and encouragement. During the period of study I had a University of London Postgraduate Studentship (1955—56, 1956—57), and an Agricultural Research Council Bursary (1957—58). I am grateful to these authorities.

ZUSAMMENFASSUNG

DIE ANFORDERUNGEN AN DAS GEWICHT DER NAHRUNG BEI EINIGEN BRITISCHEN NABIDEN (HETEROPTERA)

Versuche, in denen das Gewicht der verzehrten Beute für jedes Larvenstadium von *Stalia major* Costa bestimmt wurde, ergaben ein allgemeines Umwandlungsverhältnis der Lebendgewichtszunahme zum Trockengewicht der aufgenommenen Nahrung von 0.51. Die Umwandlungsrate änderte sich bei Temperaturen zwischen 13,5 und 22° C nicht. Eine einfache Beziehung zwischen den Anfangsgewichten der aufeinanderfolgenden Stadien, nämlich daß sie einer regelmäßigen geometrischen Progression folgen, wird für *Stalia major* aufgezeigt und zur Berechnung der mittleren Anfangsgewichte für Larven von drei anderen Nabiden-Arten (*Nabis rugosus* L., *N. flavomarginatus* Scholtz und *Dolichonabis limbatus* Dahlbom) benutzt. Die Ergebnisse werden durch Vergleich mit den Gewichten von Freilandfängen bestätigt.

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THE RELATION OF WEIGHT OF FOOD INGESTED TO INCREASE IN BODY-WEIGHT DURING GROWTH IN THE BED-BUG, *CIMEX LECTULARIUS* L. (HEMIPTERA)

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The increase in body-weight before feeding of successive instars of *Cimex lectularius* is of the order of 30% of the weight of the blood ingested at each instar, except in the 1st—2nd instars where it is approximately 40%. There is a characteristic trend in the efficiency with which the food is used to increase body-weight from instar to instar and the mean value per instar between different hosts is positively related to the proportion of solid matter in the blood. The efficiency does not vary appreciably with temperature between 20° and 30° C.

In the foregoing paper in this journal, FEWKES (1960) shows that the nabidid bug *Stalia major* Costa uses its food with great efficiency in increasing the body-weight of successive instars. Dr. K. Mellanby showed me Dr. Fewkes' manuscript and suggested that, because of the scarcity of such information for insects, I might add some of my results on the bed-bug obtained many years ago.

My old data are only for 2nd to 5th instars at one temperature although for three different hosts (man, mouse and domestic fowl). I have therefore obtained additional figures for 1st instar to adult at two other temperatures so making a more satisfactory comparison with *Stalia major*. I have also included some figures calculated from the results of JONES (1930) (Table III).

MATERIAL AND METHODS

The insects described in Table I were from a culture fed on man and kept at 23 to 25° C. Four groups of 1st instars, taken at random from a mixed batch of unfed insects 2 to 4 weeks old, were each fed once to repletion on man (C. G. J. & A. H.), mouse and domestic fowl. After moulting, the batches of each successive instar were kept for 2 days and again fed once to repletion on the appropriate host. Temperature and relative humidity were constant at 26° C and 75% during development.

The successive instars from 2nd to 5th — i.e. the same individuals — were weighed in groups: the number diminished during development, for a few bugs always refused food or suffered from that common complaint of gorged bed-bugs — ruptured gut.

New data (Table II) were obtained, from a culture from the London School

TABLE I

Weights of C. lectularius *before and after feeding: fed on man (A.H. & C.G.J.), mouse and domestic fowl; 26° C: 75% R.H.: all weights in mg.*

	Instar	II			III			IV			V
Host	no.	wt. before	wt. after	no.	wt. before	wt. after	no.	wt. before	wt. after	no.	wt. before
A.H	33	8.33	44.04	31	18.95	85.43	31	39.31	173.62	29	73.62
C.G.J.	33	8.00	36.27	27	15.25	70.34	26	32.07	139.30	25	59.37
Mouse	55	13.91	77.21	51	32.02	148.97	45	63.22	268.55	44	117.19
Fowl	47	11.72	58.21	44	24.55	116.59	37	43.36	21.72	35	81.05

TABLE II

Weights before and after feeding of C. lectularius *: fed on man. All weights in mg.*

Temp. ○ C	RH	Instar I			II			III			IV			V			Adult	
		no.	Wt. before	Wt. after	no.	Wt. before	Wt. after	no.	Wt. before	Wt. after	no.	Wt. before	Wt. after	no.	Wt. before	Wt. after	no.	Wt. before
30 ± 1.0	92	50	5.32	25.33	48	13.63	70.21	48	33.01	135.56	48	64.8	285.27	47	135.97	587.11	23	115.07
20 ± 0.5	86	50	5.23	24.64	48	12.64	67.86	48	30.54	147.49	48	66.9	308.54	44	133.39	520.50	38	196.20
27*	75	50	7.00	60.00	50	14.50	95.00	50	39.0	170.0	50	70.0	295.0	50	145.0	620.0	50	300.0

* (Jones 1930)

TABLE III

Gross Efficiency (%) of food utilization in growth of C. lectularius

	Host	Instar Temp.	I-II	II-III	III-IV	IV-V	V-Adult	Mean (weighted)	% Dry matter in blood samples
Table I	A.H.	26°	—	33.2	30.6	34.1	—	32.6	21.4
	C.G.J.	,,	—	37.6	32.8	27.7	—	33.1	21 3
	Mouse	,,	—	32.6	33.9	27.6	—	31.5	18.4
	Fowl	,,	—	31.2	29.4	24.3	—	28.6	17.2
Table II	Man	30°	42.5	34.8	31.0	33.6	22.0	—	—
	Man	20°	41.0	33.0	30.9	32.5	24.2	—	—
(Jones 1930)	Man	27°	14.2	30.4	23.7	33.3	32.6	—	—

of Hygiene and Tropical Medicine, bred on rabbit at 23° C. Eggs were incubated and hatched at 23° C and the 1st instar larvae were kept at this temperature for 2—4 days before being divided into 2 batches, weighed and fed. One lot was then kept at 30° ± 1° C (R.H. 92%) and one at 20° ± 0.5° C (R.H. 86%) but at the same saturation deficiency. Prefeeding periods of successive instars were thereafter 1—2 days for 30°, and 2—6 days for 20°, groups.

Gross efficiency was calculated from mean weights for single insects (Table III).

$$\%\ \text{efficiency} = \frac{100\ \text{(difference of mean weights of successive unfed instars)}}{\text{mean weight of blood ingested}}$$

RESULTS

Gross efficiency varied slightly between man, mouse and fowl in the same direction as the proportions of dry matter in the blood (JOHNSON, 1937): (Table III).

There was a consistent trend in efficiency from instar to instar with the maximum at 1st to 2nd instar and the minimum at 5th instar to adult: an intermediate minimum occurred at the 3rd to 4th instar: this trend is not consistently related to the relative length of instars (JOHNSON, 1942). Results from JONES' (1930) figures agree except for the first instar with which there appears to be an error in weighing; Jones however gave his insects more than the one opportunity to feed in each instar.

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ZUSAMMENFASSUNG

DIE BEZIEHUNG ZWISCHEN DEM GEWICHT DER AUFGENOMMENEN NAHRUNG UND DEM ANSTIEG DES KÖRPERGEWICHTES BEI DER BETTWANZE, CIMEX LECTULARIUS *L. (HEMIPTERA)*

Larven von *Cimex lectularius* wurden schubweise gewogen und gefüttert, wobei die Individuen in jedem Stadium eine Blutmahlzeit erhielten. Die Gewichtszunahme der — jeweils vor der Nahrungsaufnahme gewogenen — folgenden Stadien drückte sich im Prozentsatz des zugeführten Blutgewichtes aus (grober Wirkungsgrad der Nahrungsverwertung): dieser Wert variierte von 42,5% zwischen erstem und zweiten Larvalstadium bis 22,0% zwischen fünftem und Adult-Stadium. Der durchschnittliche Wirkungsgrad (Nutzeffekt) verlief vom zweiten bis zum fünften Stadium parallel zum Anteil an Trockensubstanz im Blut der verschiedenen Wirte. Im allgemeinen lag der Nutzeffekt bei 30%, aber es traten übereinstimmende Minima vom dritten zum vierten Larvenstadium und vom fünften Stadium zur Imago auf. Der maximale Wirkungsgrad lag im ersten Larvenstadium. Temperaturen zwischen 20 und 30° C waren ohne merklichen Einfluß auf die Nahrungsausnutzung.

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FOOD PLANT RELATIONSHIPS OF THE DIAMOND-BACK MOTH *(PLUTELLA MACULIPENNIS* (CURT.))

I. Gustation and Olfaction in Relation to Botanical Specificity of the Larva

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The exclusion of botanical species from the food plant range of the diamond-back moth larva is determined not only by the infrequency of adequate stimulants for feeding but also by a high frequency of occurrence of feeding inhibitors. A few plant species lack feeding inhibitors but are excluded by a deficiency of feeding stimulants. Some plants are devoid of decisive feeding inhibitors and contain effective feeding stimulants but yet are excluded as host plants by the possession of toxic principles. At least one plant species, *Pisum sativum*, devoid of mustard oil glucosides, is sufficiently palatable, nutritious and free of toxicants to support successive generations of *Plutella* larvae in the laboratory. The free mustard oils in the natural host plants apparently tend to inhibit dispersal of the larvae.

The food plant range of the diamond-back moth, *Plutella maculipennis* (Curt.) is essentially coincident with the botanical distribution of the mustard oils and their glucosides (THORSTEINSON, 1953). The gustatory influence of mustard oil glucosides in the food plants was clearly shown to provide an essential stimulus for ingestion and to be a significant determinant of food plant specificity insofar as this is determined by the behaviour of the larvae.

Preliminary experiments indicated that the occurrence of feeding inhibitors as well as the absence of essential feeding stimulants excluded some botanical species from the food plant range. Feeding inhibitors could exercise a decisive veto against acceptability of plants as food even if the plants possessed all the stimulants necessary to elicit feeding. These inhibitory factors are therefore potentially as significant for food plant specificity as are feeding stimulants. The authors have consequently investigated the botanical occurrence of such feeding inhibitors more extensively.

The existence in plants of effective feeding stimulants other than mustard oil glucosides is a possibility that requires consideration. If they exist, they can be detected conveniently only in plants that do not contain feeding inhibitors. Such feeding stimulants apparently do occur in some plants devoid of mustard oil glucosides especially among the Leguminosae, as will be demonstrated later.

The nutritional adequacy of such plants therefore becomes open to investigation and is considered in a later section. The fact that these apparently susceptible plants are not attacked in nature is explained in a separate paper by the behaviour of the adult female (GUPTA & THORSTEINSON, 1960).

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Although the free mustard oils do not contribute significantly to feeding responses as such (THORSTEINSON, 1953), the possibility remains that these odorous substances may indirectly contribute to the mechanism of food plant specificity. For example, the odor of these essential oils may inhibit dispersive tendencies and prevent the larvae from wandering away from the food plant. This will be considered in the experiments on olfaction in a later section. The question of attraction normally does not arise, at least at the beginning of the life of larvae which hatch from eggs deposited on the food plant by the female parent. The literature of olfactory attraction in larvae indicates that attraction is effective over distances of only a few millimetres (GÖTZ, 1936; CHIN, 1950).

MECHANISMS OF FOOD PLANT EXCLUSION

As indicated previously, a full understanding of the chemical basis of food plant specificity requires consideration of chemical plant constituents that inhibit feeding. These are to be found, if anywhere, among plants outside the food plant range of the insect. The presence of such inhibitors is easily detected for certain insects by the method originated by VERSCHAFFELT (1910) in his study of food plant specificity of *Pieris* spp. and extended later to the investigation of food plant relationships of *Plutella* (THORSTEINSON, 1953). The application of this procedure to a large number of plant species normally unacceptable to *Plutella* larvae provides a basis for evaluating the generality of the significance of feeding inhibitors as determinants contributing to food plant specificity.

In the present investigations, 62 species of plants, none possessing mustard oil glucosides and belonging to 37 different families, were offered to third and fourth instar larvae. Six discs, 12 mm in diameter, were cut from fresh leaves of each plant species grown in the green house. Three discs were placed in each of two plastic dishes. The leaf discs in one series of dishes were treated with 2% sinigrin solution, while the leaf discs in the other series were left untreated. Five larvae were used in each dish and at least 4 replicates were made. The temperature ranged between 23.3—26.7° C and the dishes were kept in the dark in desiccators containing water. The discs were exposed to the larvae for 18 hours and control discs of black mustard leaf were maintained. The results are given in Table I.

Nine of the 62 plant species tested, namely *Lactuca sativa, Melilotus alba* var. *coumino, M. officinalis, Medicago sativa, Trifolium pratense, Vicia faba, Pisum sativum, Allium cepa* and *Phlox adsurgens* were ingested to an appreciable degree although none of these contain mustard oil glucoside. Palatability, however, did increase when the leaf discs were painted with sinigrin. Six of these species belong to the family Leguminosae and all of these seem to contain substances possessing a chemosensory influence simulating that of mustard oil glucosides (possibly exciting some of the same receptors) and apparently are devoid of decisive feeding inhibitors for *Plutella* larvae.

TABLE I

Feeding Responses of Diamond-back Moth Larvae to Non-host Plants

Family	Plant	Untreated leaf discs	Leaf discs treated with sinigrin
Acanthaceae	*Fittonia argyroneura* E. Coem.	—	—
	F. verschaffeltii E. Coem.	—	—
Apocynaceae	*Nerium oleander* L.	—	—
Araliaceae	*Hedera helix* L.	—	—
Aroideae	*Philodendron sp.*	—	—
Buxaceae	*Buxus sempervirens* L.	—	—
Begoniaceae	*Begonia pearcei* Hook.	—	—
Caryophyllaceae	*Dianthus sp.*	—	—
Celastraceae	*Euonymus japonicus* L.	—	—
Commelinaceae	*Tradescantia sp.*	—	—
Compositae	*Senecio cineraria* DC	—	—
	Lactuca sativa L.	++	+++
	Dahlia sp.	—	++
	Gynura sp.	—	+
	Chrysanthemum sp.	—	+
Convolvulaceae	*Ipomoea batatas* Poir.	—	—
Cucurbitaceae	*Cucumis sativus* L.	—	++
Ericaceae	*Azalea sp.*	—	—
Euphorbiaceae	*Euphorbia lathyris* Georgi	—	—
	E. poinsettiana Buist.	—	+
	E. plendens Boj.	—	+
Gentianaceae	*Exacum sp.*	—	—
Geraniaceae	*Geranium sp.*	—	—
	Impatieus sultani L.	—	—
	Pelargonium sp.	—	—
	Oxalis deppei Lodd.	—	—
Gesneriaceae	*Naegelia hyacinthi* Carr.	—	—
Labiatae	*Coleus sp.*	—	—
Leguminosae	*Melilotus alba*, var. *coumino* Desr.	+++	++++
	M. officinalis Lam.	++	++++
	Medicago sativa L.	++	+++
	Trifolium pratense L.	++	+++
	Vicia faba L.	+	++
	Pisum sativum L.	++	++++

TABLE I (continued)

Family	Plant	Untreated leaf discs	Leaf discs treated with sinigrin
Liliaceae	*Allium cepa* L.	+	++
	Tulipa sp.	—	—
	Lilium longiflorum Thunb.	—	—
	Hemerocallis dumortieris Morr.	—	—
Lythraceae	*Punica granatum* St. Lag	—	—
Malvaceae	*Hibiscus syriacus* L.	—	—
	Abutilon pictum Walp.	—	+
Marantaceae	*Maranta bicolor* Ker. Gawl.	—	++
Onagraceae	*Fuchsia sp.*	—	—
Orchidaceae	*Bletia hyacinthiana* R. Br.	—	—
Passifloraceae	*Passiflora alata* Dryand.	—	—
Piperaceae	*Peperomia sp.*	—	+
Polemonaceae	*Phlox adsurgens* Torr.	+	++
Rhamnaceae	*Rhamnus crenata* Sieb. & Zucc.	—	+
Ranunculaceae	*Delphinium chinense* Fisch.	—	—
	Clematis sp.	—	+
Rosaceae	*Rosa sp.*	—	++
Rutaceae	*Citrus aurantium* L.	—	—
Saxifragaceae	*Hydrangea sp.*	—	—
	Heuchera sanguinea Engelm.	—	—
Solanaceae	*Petunia sp.*	—	—
	Solanum tuberosum L.	—	—
	Solanum sp.	—	—
	Lycopersicum esculentum Mill.	—	—
Urticaceae	*Pellionia pulchra* N. E. Br.	—	—
	Ficus carica L.	—	—
Verbenaceae	*Lantana camara* L.	—	—
Vitaceae	*Cissus rhombifolia* Vahl.	—	—

Twelve plant species, normally refused, were accepted when treated with sinigrin; these include *Dahlia* sp., *Gynura* sp., *Chrysanthemum* sp., *Cucumis sativus, Euphorbia poinsettiana, E. splendens, Abutilon pictum, Maranta bicolor, Peperomia* sp., *Rhamnus crenata, Clematis* sp. and *Rosa* sp. As is clear from Table I, these belong to different families and there is no apparent botanical affinity among them. Evidently, these species are naturally deficient in essential feeding stimulants but contain relatively weak feeding inhibitors or none.

About two thirds of the species of plants investigated remain unacceptable to the larvae even when treated with sinigrin. The following hypotheses suggest themselves:

(1) these plants are deficient in one or more substances other than mustard oil glucoside which are essential feeding stimulants for *Plutella* larvae.

(2) these plants contain all the essential feeding stimulants other than mustard oil glucoside but contain also substances that inhibit feeding responses ("veto" the sinigrin stimulus).

We have, at present, no evidence to support the first hypothesis and it appears justifiable to accept the second, *viz.*, that the majority of unacceptable plant species contain chemosensory feeding inhibitors that render them unacceptable to *Plutella* larvae. Food plant specificity may therefore be determined for an oligophagous insect by the botanical distribution of feeding inhibitors as well as essential feeding stimulants.

There is no logical basis for considering the presence of an inhibitor as a less significant circumstance than the absence of an essential feeding stimulus as an exclusion mechanism in relation to food plant range of an insect. Indeed we must admit the possibility that some of the rejected plants may naturally possess all of the essential feeding stimulants (including something that could provide the effect of sinigrin) and would be acceptable if the inhibitor were not present. Unfortunately this possibility is difficult to put to an unequivocal test but this concept has been invoked by students of the feeding behaviour of *Leptinotarsa decemlineata* (Say), implicitly by LANGENBUCH (1952) and explicitly by JERMY (1958).

That these inhibitors are chemicals rather than physical attributes was demonstrated by treating mustard leaf discs with methanol or aqueous extracts of rejected plants. Only slight feeding occurred on mustard leaf discs treated with petunia extract and only moderate feeding on those treated with tomato leaf extract. No feeding inhibition occurred on mustard leaves treated with extracts of leaves of pea, sweet clover and coumino clover. Similar results were obtained when extracts of these were applied to thin slices of Japanese elder pith treated with sinigrin.

DEVELOPMENT OF THE LARVA ON PLANTS DEVOID OF MUSTARD OIL GLUCOSIDES

The observations mentioned earlier with regard to the larvae feeding in appreciable quantity on leaves of certain species of leguminous plants suggest that, under laboratory conditions, the larvae may be capable of sustaining themselves on some of the plants which do not contain mustard oil glucosides. This possibility was investigated.

Within 12 to 16 hours of hatching on leaves of black mustard, the larvae were transferred to healthy plants of garden pea, sweet clover, coumino clover, red clover, alfalfa, broad bean and tomato. About 30—35% of the larvae placed on the first three plant species established themselves on the leaves and appeared to thrive while the others either died of starvation or left the plant. Red clover and alfalfa did not appear to elicit continued feeding and no live larvae remained on these plants after five days. Broad bean and tomato did not elicit any feeding and all the larvae died by the second day.

As the larvae placed on the pea, sweet clover and coumino clover plants developed, they tended to disperse and by the time they reached the fourth instar most of them were lost. However, about 5% of these larvae remained on the plants till completion of development and pupated on the leaves within an average period of 15 days from the date of hatching. Larvae confined in plastic bags wrapped around leaves did well in the early part of their development but after 7—8 days most of them died, probably due to bacterial or fungal disease.

Attempts were also made to rear the larvae on pea, sweet clover and coumino clover leaves placed in covered paper cartons or plastic dishes to prevent escape of the larvae. Old leaves were removed and replaced by fresh leaves twice a day. High humidity was maintained by keeping the cartons and dishes in a desiccator containing distilled water. About 60% of the newly hatched larvae transferred from mustard to any of the legume species died within two days while the majority of the larvae reared throughout on mustard survived. The rate of mortality declined as the larvae grew older and about 32% reached the fourth instar stage on pea leaves, and about 26% on the sweet clover and coumino clover leaves. At this stage several larvae showed symptoms of a disease not previously seen in our cultures. They became sluggish, black streaks appeared in the posterior third of the body and the anal aperture became obstructed by a faecal plug and they died within two or three days. Only about 14% of the total number of larvae survived to the pupa stage as compared with 53% in the controls reared on mustard. It is of interest that larvae reared throughout on mustard leaves do not succumb to the disease described above. Conceivably, mustard leaves contain some therapeutic constituent.

The larvae reared on legumes as described above had had a brief opportunity to feed on mustard during a few hours after hatching. Experiments were arranged to determine whether larvae could develop on these legumes if they had no opportunity to feed on natural food plants at any time. Untreated, rugose plastic caps, on which *Plutella* moths had oviposited, were placed with leaves of garden pea, sweet clover or coumino clover in rearing containers. The larvae hatched as usual in five to six days and moved to the leaves. As in earlier experiments, fresh leaves were changed twice a day and drying of leaves was reduced by high humidity. Unlike the result in the earlier experiments, none of the larvae maintained on either sweet clover or coumino clover survived beyond 3—4 days. The use of sweet clover and coumino clover leaves treated with sinigrin solution did not improve survival. On the other hand, approximately 30% of the larvae provided with pea leaves thrived to the fourth instar when some died of the disease mentioned above and ultimately, 17% successfully pupated.

Adults emerged from almost all the pupated larvae raised on pea, sweet clover and coumino clover leaves in these experiments and included both males and females. These moths were kept separate from the stock culture for observation. No differences could be detected in either morphology or behaviour as compared with moths reared from mustard. They laid appreciable numbers of viable eggs on rugose plastic discs and on leaves of black mustard but not more than 2 or 3 eggs were laid on pea, sweet clover and coumino clover leaves placed in the cages. Our observations provide no support for the operation of the Hopkins Host Selection Principle (HOPKINS, 1917).

Larvae from these eggs could be reared on sweet clover and coumino clover leaves to complete development as before provided they were allowed to feed on mustard leaves for 10—12 hours after hatching. On the other hand, four generations were continuously raised on pea leaves without ever using cruciferous or other plants containing mustard oil glucosides either for feeding the larvae or for oviposition by the adults. The fact that *Plutella* populations do not develop on pea plants in nature will be understood in the light of the oviposition behaviour of this insect to be described in a separate paper (GUPTA & THORSTEINSON, 1960).

The results of these experiments are of interest in that although there is sustained feeding on five of six species of leguminous plants investigated, only three species, *viz.*, garden pea, sweet clover and coumino clover approach nutritional suitability for *Plutella* larvae. The physical character of the broad bean leaf is different from that of the other species and this may have been responsible for preventing the young larvae from feeding on it. The leaves of red clover and alfalfa, however, appear similar in texture to those of the sweet clover and coumino clover. The death of the larvae fed on these two species may be attributed to either the lack of essential nutrients or the presence of toxic principles or both since preliminary experiments indicated that the palatability of these plants appears adequate, and it is noteworthy that treating the leaves of these plants with sinigrin to correct deficiency of feeding stimulant did not improve survival.

Development was successful on sweet clover and coumino clover only if the larvae had been "nursed" through part of the first instar on mustard leaves but this precaution was not necessary for rearing larvae on pea leaves. Possibly, first instar *Plutella* larvae are susceptible to toxic factors in *Melilotus* leaves to which the later instars are more resistant. Alternatively, *Melilotus* leaves may be deficient in some nutritional factor peculiarly essential to the survival of the young larvae.

THE ROLE OF FREE MUSTARD OILS

The food plants of the diamond-back moth contain small amounts of mustard oils in the free form as well as in glucosidal combination. These essential oils typically have very pungent odors and it is of interest to consider whether they affect food-plant specificity. The role of these mustard oils in the behaviour of adult *Plutella* is treated in another paper (GUPTA & THORSTEINSON, 1960). Their influence as feeding stimulants *per se* was shown previously to be negligible (THORSTEINSON, 1953). It remains to consider whether mustard oils play some indirect role, for example as attractants, or otherwise, in the food plant relationships of the diamond-back moth larva.

Preliminary screen tests of the type introduced by DETHIER (1937) indicated that bruised mustard leaves exert a significant olfactory influence on the spatial distribution of *Plutella* larvae but it was not determined whether this effect was attributable to the mustard oils, humidity or other vapours emanating from the leaves. These experiments were therefore repeated, replacing leaves with pure allyl isothiocyanate and with water controls.

In each trial, the test substance contained in a small watch glass was placed in a plastic dish, 15 cm in diameter, 1.0 cm below the screen and 0.5 cm from the periphery. Larvae were introduced either directly above the odour source (experiment A) or at the opposite end at a distance of 14 cm (experiment B). The dishes were kept for 6—14 hours in the dark. The variation in the duration of the tests did not appear to affect the results. The combined data are presented in Table II.

TABLE II

Mean Numbers of Larvae by Distance from Odor Source in Screen Tests*

Distance from Odor source in cms.	Larvae Introduced Directly Above Odor Source		Larvae Introduced at Opposite End 14 cm from Odor Source	
	10 p.p.m. aqueous solution of allyl isothiocyanate	Distilled water	10 p.p.m. aqueous solution of allyl isothiocyanate	Distilled water
0— 1	2.06	0.91	1.34	0.94
1— 2	1.28	0.98	1.53	0.94
2— 3	1.07	0.86	1.24	1.13
3— 4	0.93	1.40	1.32	1.03
4— 5	0.83	1.03	0.94	1.05
5— 6	1.05	1.16	1.03	1.19
6— 7	0.78	1.03	0.88	1.15
7— 8	1.05	0.98	0.98	0.99
8— 9	1.25	1.09	0.94	1.09
9—10	0.93	0.93	1.11	1.07
10—11	1.00	1.05	0.94	1.23
11—12	0.93	1.51	1.11	1.09
12—13	1.12	1.00	1.11	1.09
13—14	0.06	1.10	1.13	1.35
14—15	0.71	1.05	0.82	1.07
	$P<1\%$	$P>5\%$	$P<1\%$	$P>5\%$
L.S.D. 1%	0.34		0.34	
5%	0.32		0.24	

* Transformation $\sqrt{x + 0.5}$ No. of larvae per test = 12. No. of Replicates = 7.

The interpretation of the data in Table II requires that we take account of the fact that aggregations of larvae were experimentally imposed — above the odor source in experiment A and 14 cm distant from the odor source in experiment B. In both experiments, these imposed aggregations decompose when there is no plant odor present other than water vapour. In experiment A with allyl isothiocyanate odor, the aggregation is reduced through dispersal of some of the larvae but the number that remain localized at or near the locus of introduction significantly exceeds the density to be expected on the basis of random distribution. It appears that the odor of allyl isothiocyanate tends either directly or indirectly to inhibit dispersal. The fact that considerable dispersal does take place is at least in part a result of the tactile stimulation hardly avoidable in the manipulation necessary to introduce the larvae into the test chamber. *Plutella* larvae are peculiarly sensitive to such tactile stimuli. This attribute might also tend to preclude any dense aggregation such as was found by WATANABE (1958) in his screen tests with *Bombyx mori* larvae.

In experiment B, the imposed aggregation 14 cms distant from the source of allyl isothiocyanate odor became decomposed through dispersal and a new aggregation was formed near the odor source. Although this aggregation is statistically significant, it is less dense immediately above the odor source and occupies a greater area than the aggregation in experiment A.

It is clear that *Plutella* larvae are influenced by the odor of allyl isothiocyanate, a characteristic constituent of its natural food plants. If this influence is one of attraction, it is only imperfectly operative under the conditions of the experiment. In experiment A, the dense, imposed aggregation does not become fully reconstituted after the initial partial dispersal induced by manipulative disturbance. In experiment B, a significant but weak aggregation is formed near the odor. Other investigators, for example CHIN (1950) have found that an overt directive locomotory response to food plant odor occurs only at distances of 5 mms or less from the leaf.

Presumably *Plutella* larvae can be attracted from distances of the order of 5 mm as is the Colorado beetle larva (CHIN, 1950). If *Plutella* larvae were attracted at greater distances, say 5—10 cms., we would expect aggregations of greater density near the odor source. As this did not occur we infer that the attractive effect, if any, of allyl isothiocyanate does not extend much beyond the distance observed by CHIN (1950). On this assumption, it follows that the direction of movements of the larvae in experiment B were random with respect to the odor source throughout the greater part of the area of the chamber. The failure of the dispersed larvae to reassemble close to the odor source in experiment A may be accounted for in the same way.

It is an attractive hypothesis that a food plant must supply some stimulus that tends to inhibit dispersal of insects that feed on it (cf. KENNEDY, 1958). In order to account for the degree of aggregation observed it is only necessary to postulate that the larvae that chance to arrive close enough to the odor source become inhibited in respect of dispersal activity. In this sense mustard oil odor might be regarded as an arrestant as defined by DETHIER, BARTON BROWNE & SMITH (1960).

The failure of the larvae to assemble in more dense aggregations near the odor source in these experiments may be due to one of the following possibilities:

1. The threshold distance for our postulated locomotory inhibition by allyl isothiocyanate is localized near the odor source and many of the larvae do not chance to enter this area during the period of the experiment.
2. The odor thresholds of the larvae vary considerably and the concentration of mustard oil vapour throughout an appreciable area of the test chamber exceeds the "arrestant threshold" for at least some of the larvae. The larvae located at appreciable distances from the odor source have low thresholds, those localized near the odor source have high thresholds.

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RESUME

L'exclusion d'espèces botaniques comme plantes pouvant servir de nourriture à la larve de *Plutella maculipennis* est detérminée non seulement par la râreté de stimulants nécessaires pour que l'insecte se nourrisse mais aussi par la présence fréquente de substances qui empêchent l'insecte de se nourrir. Quelques espèces de plantes qui n'ont pas de substances qui empêchent l'insecte de se nourrir, sont excluées par une déficience de stimulants. Certaines plantes n'ont pas de substances qui empêchent l'insecte de se nourrir et contiennent des stimulants efficaces, mais cependant elles sont excluées comme plantes nourricières par la possession de principes toxiques. Au moins une espèce de plante, *Pisum sativum*, qui ne possède pas les glucosides d'huile de moutarde, est assez agréable au goût, nourrissante et privée de toxines pour pouvoir soutenir des générations successives de larves de *P. maculipennis* dans la laboratoire. Les huiles de moutarde des plantes nourricières naturelles semblent empêcher la dispersion des larves.

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THE LOCATION OF HYGRORECEPTORS AND MOISTURE RECEPTORS IN AEDES AEGYPTI (L.)

BY

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Hygroreceptors which mediate dry or wet reactions are located on the antennae. Moisture receptors which mediate dry or wet reactions are located on the tarsi. The palpi do not seem to possess hygroreceptors.

Tarsiless mosquitoes gave a different response to moisture than intact mosquitoes or mosquitoes with covered tarsi.

The activity of antennectomized mosquitoes was considerably reduced, whereas the activity of mosquitoes with covered antennae was only moderately reduced. The activity of mosquitoes with covered or removed tarsi was not affected.

The literature relating specifically to hygroreception in arthropods is not large. Most investigators have demonstrated that hygroreceptors of one type or another are located mostly on various parts of the antennal segment. (Pielou & Gunn, 1940; Wigglesworth, 1941; Bentley, 1944; Begg & Hogben, 1946; Roth & Willis, 1951a & b; Anderson & Ball, 1959). In some cases other parts of the body were also found to be hygroreceptive. Gunn (1937) detected hygroreception on the thoracic region of a wood louse (*Porcellio scaber* Lat.), Lees (1948) in the anterior pit of Haller's organ of a tick (*Ixodes ricinus* L.), Bursell & Ewer (1950) all over the body surface as well as on the antennae of an onychophoran (*Peripatopsis moselyi*), Lees (1943) on the antennae and on the maxillary and labial palpi of a wireworm (of the genus *Agriotes* Esch.). In mosquitoes, Roth & Willis (1952) observed that the antennae in *A. aegypti* are hygroreceptively active. Roth (1951) concluded that the antennae and palpi are the chief organs used by *A. aegypti* females in locating the host and receiving the stimuli which induce probing. Rahm (1958) found that the antennae of *A. aegypti* were greatly responsible for the attraction to a warm wet flask. Unsuccessful attempts to identify the sites of hygroreceptive activity have been made by Thomson (1938) on the mosquito *Culex fatigans*. The present paper is an attempt to localize the hygroreceptors and possible moisture receptors of the female *Aedes aegypti* (L.).

MATERIALS AND METHODS

The method consisted of removing, or covering with an impervious material, certain organs of the mosquito suspected to bear hygroreceptors and moisture receptors, and determining the reaction of such mosquitoes to a wet surface and to the air close to it by the method described in a previous paper (Bar-Zeev, 1960).

Removal of antennae, palpi and tarsi: The method of removing the antennae or palpi was as follows: The mosquitoes were kept in a small cup on ice to render them inactive. A mosquito was then placed with the dorsal side of its thorax on the opening of a capillary tube (90 mm outside diameter, 0.6 mm inside diameter) held under a binocular, and connected to a water jet pump to hold the mosquito firmly in this position. Light from a microscope lamp passed through the mirror of the binocular into the capillary tube illuminating the mosquito satisfactorily. The antennae or palpi were removed with a very fine tweezer (Genuine Th. Dumont tweezer, Switzerland). After removal of the flagellum the edge of the pedicel was covered by means of a fine needle with silicon grease (W. Edwards & Co. London Ltd.) to ensure that any tiny fragment of the first flagellar segment that might have remained would be covered with grease. The mosquito was then sucked into a glass tube (1 cm in diameter), at the same time releasing the vacuum, and transferred to a beaker which contained a cotton wad soaked in a 10% sugar solution. The mosquitoes were used 24 hours after the operation.

The tarsi were removed by placing the mosquito on a white blotting paper, and cutting of the tarsi with a fine scalpel.

Coverage of the tarsi and antennae. For covering the tarsi, a mixture of solid and liquid paraffin oil was used. The solid paraffin was melted, and 50% (by weight) of liquid paraffin was added to it, in order to lower its melting point. Such a mixture does not solidify very fast when brought to the room temperature, and, therefore, covering is rendered easier. For covering the antennae, however, this mixture was not suitable since, relatively, much more time is required to cover the antennae than the tarsi. For covering the antennae warmed silicon grease (W. Edwards & Co. London) proved more suitable. The antennae and tarsi were covered with a fine artist brush, and the covered parts were examined under a binocular to see if coverage was complete.

THE LOCATION OF THE HUMIDITY AND MOISTURE RECEPTORS

It has been shown that in some arthropods two types of receptors may be present which mediate a wet and dry reaction, respectively. These two types of receptors may be located on different parts of the body of the animal (BEGG & HOGBEN, 1946; BURSELL & EWER, 1950; ROTH & WILLIS, 1951b) or on the same appendage (ROTH & WILLIS 1951b, 1952). In the following experiments the location of receptors which mediate a dry and wet reaction, was attempted.

The location of receptors which mediate a dry reaction. Results of variously treated mosquitoes are given in Table I.

It can be seen, from results obtained on the surface, that antennectomized, as well as antennectomized and palpectomized mosquitoes, still gave a significant dry reaction, although greatly reduced as compared with that of intact mosquitoes. The same is true with mosquitoes with covered antennae or covered tarsi, whereas mosquitoes with covered antennae *and* tarsi gave no reaction. From the results obtained close to the surface, it will be seen that antennectomized mosquitoes or those with covered antennae gave no significant reaction, whereas intact mosquitoes gave a moderate dry reaction. It may, therefore, be concluded that hygroreceptors and moisture receptors which mediate a dry reaction are located on the antennae and tarsi, respectively.

TABLE I

The reaction of variously treated mosquitoes to a wet surface and to the air close to it

	On the surface			*Close to the surface*		
Type of treatment	Number of replicates	N	I.R. ± S.E.	Number of replicates	N*	I.R. ± S.E.**
Intact	12	1761	93.1 ± 1.5	6	2711	41.6 ± 3.2
Antennectomized	12	4632	20.4 ± 4.7	6	2847	0.9 ± 2.6
Antennectomized & palpectomized	12	4586	21.4 ± 4.1			
Covered tarsi	10	1935	31.2 ± 1.4			
Covered antennae	12	2364	30.9 ± 4.5	6	2591	2.3 ± 3.8
Covered antennae and tarsi	12	3743	—1.4 ± 4.6			

* N = Total number of mosquitoes counted.

** I.R. ± S.E. = Index of reaction ± standard error (see Bar-Zeev, 1960, p. 200).

The intensity of the reaction of antennectomized compared to that of antennectomized *and* palpectomized mosquitoes is similar, indicating that the palpi do not bear hygroreceptors which mediate a dry reaction.

Experiments to determine the response on the surface with tarsiless mosquitoes were also carried out. Perplexing results were obtained. If the experiments were carried out about two hours after removal of the tarsi, a moderate wet reaction was obtained. (I.R. = —26.8, 10 replicates. Total number of mosquitoes counted (N) = 4362). If the tarsiless mosquitoes were tested 24 hours after removal of the tarsi, no reaction was obtained (I.R. = 1.6, 10 replicates, N = 4320).

The location of receptors which mediate a wet reaction. In order to locate the receptors which mediate a wet reaction, similar experiments, as previously, were carried out with desiccated mosquitoes (2 hours over $CaCl_2$ in a sealed desiccator) the proboscis of which had been removed (to prevent drinking). Results are given in Table II.

TABLE II

The reaction of variously treated desiccated mosquitoes to a wet surface and to the air close to it

	On the surface			*Close to the surface*		
Type of treatment	Number of replicates	N	I.R.	Number of replicates	N	I.R.
Intact	6	1379	—67.9 ± 6.6	6	2686	—24.0 ± 2.5
Antennectomized	10	4234	—25.6 ± 6.4	6	2847	0.9 ± 2.6
Covered antennae	6	2459	—52.0 ± 5.0	6	2700	2.6 ± 3.3
Covered tarsi	6	2432	—51.7 ± 5.2			
Covered antennae and tarsi	6	2538	1.5 ± 4.8			

It will be seen from results obtained on the surface with antennectomized mosquitoes, insects with covered antennae or covered tarsi, that a significant though reduced wet reaction was still obtained, as compared to the behaviour of intact desiccated mosquitoes, whereas mosquitoes with covered antennae and tarsi gave no reaction. Results obtained close to the surface show no reaction of mosquitoes with covered antennae or antennectomized insects, whereas intact mosquitoes gave a moderate wet reaction. It may, therefore, be concluded that hygroreceptors and moisture receptors which mediate a wet reaction are located on the antennae and tarsi, respectively.

THE RELATIVE ACTIVITY OF MOSQUITOES FROM WHICH VARIOUS ORGANS WERE REMOVED OR COVERED

It was observed during the course of the present investigation that the activity of antennectomized mosquitoes was greatly reduced. WIGGLESWORTH & GILLET (1934) showed that the antennae of *Rhodnius prolixus* have a kinetic function, and normally keep the nervous system in a state of "tone" in which it will respond readily to various stimuli. PIELOU & GUNN (1940) claim that the activity of *Tenebrio molitor* did not seem to be affected by antennectomy.

It was of interest to determine the relative activity of the variously treated groups. The method used was as follows: Eight mosquitoes were placed in a petri dish similar to that used previously, the bottom of which was lined with dry filter paper, and the dish placed in the apparatus described. Every minute or so, the lever of the apparatus was tapped once (to jar the petri dish) inducing some mosquitoes to fly. The number of mosquitoes in flight was recorded. An empirical measure of relative activity was thus derived. To prevent fatigue, only 10 such counts were taken, and then another group was tested. A total of 80 mosquitoes was tested in each experiment. The per cent of mosquitoes thus activated is given in Table III.

TABLE III

Percentage of variously treated mosquitoes activated by a standard jar

Type of treatment	Per cent activated
Intact	60.1
Antennectomized	4.0
Antennae covered	37.7
Tarsi covered	62.6
Tarsi & antennae covered	35.9
Tarsi removed	57.2
Desiccated, proboscis removed, antennae & tarsi covered	33.7

It can be seen that the activity of antennectomized mosquitoes was considerably reduced as compared with intact mosquitoes or with covered antennae. The activity of the latter was also moderately reduced, whereas mosquitoes with tarsi removed or covered do not seem to be affected. It can be seen (Table I and II) that antennectomized mosquitoes gave a weaker response than mosquitoes with covered antennae. This is most probably due to the fact that the removal of the antennae abolished their kinetic effect.

It can be seen (Tables I, II) that the total number (N) of antennectomized mosquitoes on the surface was always higher than that of intact or otherwise treated mosquitoes, although equal numbers of mosquitoes were used. As only mosquitoes standing *on* the surface are counted, antennectomy causes many more mosquitoes to stand on the surface.

DISCUSSION

The intensity of response of intact mosquitoes (normal or desiccated) was

much stronger *on* the surface than close to it. (Table I and II). This difference in response may be due to two factors:
a) The R.H. just at the wet surface is probably higher than that close to it. Various investigators working on different insects, have shown that the wetter the alternative, the more intense the reaction (PIELOU & GUNN, 1940; LEES, 1943; BENTLEY, 1944; WILLIS & ROTH, 1950; SMEREKA & HODSON, 1959).
b) The combined effect of hygroreceptors and moisture receptors is stronger than the separate effect of either group. The dry reaction of intact mosquitoes (Table I), or the wet reaction of intact desiccated mosquitoes (Table II) was much stronger than the reaction of mosquitoes with covered antennae, covered tarsi or without antennae. It appears that the second factor is the more important, since ROTH & WILLIS (1952), comparing the effect, on female *A. aegypti*, of 100% R.H. to 75% R.H. in an olfactometer (which evidently does not affect the moisture receptors on the tarsi) obtained only a moderate dry reaction (I.R. = 51 ± 4.6), similar to the results close to the surface, obtained by the method used in the present investigation.

Tarsiless mosquitoes gave no reaction when tested 24 hours after removal of the tarsi, and gave a moderate wet reaction when tested about two hours after removal of the tarsi (page 253). These results are not in agreement with results obtained on mosquitoes in which the tarsi were covered (which gave a moderate dry reaction). No explanation is offered for this phenomenon. Somewhat similar observations were made by BENTLEY (1944) and BEGG & HOGBEN (1946). The former found that removing part of the antennae of *Ptinus tectus* turned the dry into a wet reaction. However, no experiments were carried out to determine the reaction of the insects in which the same part of the antennae was covered. Begg & Hogben have shown that the reaction to humidity of an antennaless mutant of *Drosophila melanogaster* was inverse to that of the same stock possessing antennae. However, no experiments were done to determine the reaction of insects possessing antennae in which the antennae were covered or removed. A difference in response due to removal of the tarsi of the wood nymph butterfly was observed by FRINGS & FRINGS (1959); tarsiless insects showed a far greater response (extension of proboscis) when the tips of the antennae were touched with sucrose, as compared with intact insects.

The different reaction obtained with tarsiless mosquitoes as compared with mosquitoes with covered tarsi, the decreased activity and, therefore, decreased response of antennectomized mosquitoes, and the difference in response due to extirpation of various organs, observed on other insects — emphasize the importance of carrying out control experiments (covering the parts to be extirpated) in order to determine a possible change in reaction.

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RESUME

LOCALISATION DES HYGRORÉCEPTEURS ET DES RÉCEPTEURS DE MOITEUR CHEZ AEDES AEGYPTI *L.*

Les organes hygrorécepteurs, qui transmettent des réactions sèches ou humides, sont situés

sur les antennes. Les récepteurs de „moiteur", qui transmettent des réactions sèches ou moites, sont situés sur les tarses. Il ne semble pas qu'il y ait des organes hygrorécepteurs sur les palpes.

La réaction à la moiteur des moustiques sans tarses est différente de celle des moustiques intacts et de celle des moustiques dont les tarses sont couverts.

L'activité des moustiques privés de leurs antennes est considérablement réduite, tandis que la réduction d'activité chez les moustiques dont les antennes ont été couvertes n'est que modérée. Couvrir ou enlever les tarses des moustiques n'a aucun effet sur leur activité.

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THE SETTLING OF FEMALE *HAEMATOPOTA INSIDIATRIX* AUSTEN (DIPTERA, TABANIDAE) ON CLOTH SCREENS

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The response of female *Haematopota insidiatrix* towards cloth screens is considered as part of the host-finding behaviour. When black, dark grey, light grey and white cloths were compared as regards attractiveness, more flies settled on the black than on the other shades. When a screen was carried horizontally flies settled on the under surface but not on the upper surface and more flies were caught. A black screen with three white stripes was progressively less attractive as the width of these stripes was increased. Even narrow white stripes reduced the catch, particularly if they were horizontally arranged. When differently sized black screens were compared, fewer flies were caught on the smaller ones. Flies settled on the lower part of a screen, as they do on a host animal. When a screen was divided so that its lower half was white and its upper half black, fewer flies settled on it than on the all-black reverse side (control). Conversely, with black below white approximately the same number of flies were caught on this as on the black control side of the screen. At temperatures below 19° C and humidities above 77% R.H., more flies settled on the sunny than on the shaded side of the screen. At higher temperatures and lower humidities this preference was reversed.

Preliminary experiments with cloth screens (BARRASS, 1959) were undertaken during a visit to the Main Camp of Wankie National Park, Southern Rhodesia, during January 1959. This work was continued in December 1959 and January 1960; the present paper refers to the two visits. The experiments are concerned with the settling of female *Haematopota insidiatrix* Austen on black, dark grey, light grey, white, and on black and white cloth screens.

METHODS

Screens were carried through the bush. Flies settling were collected by one person on each side of the screen and a stop was made at regular intervals (50 or 100 yds, = 45 or 90 m) for this purpose. Flies were collected in tubes of 7.5 x 2.5 cm so that other flies on the screen were not disturbed. Only the blood-sucking females settled on the screens and only these were studied. The screen was rotated after each stop to avoid bias caused by differences in the collectors' ability, by the direction of the sun or by differences in vegetation on the two sides. Four shades of cloth were used which all had the same texture. The relative intensities of light reflected from the cloths were measured in a dark room with an EEL Lightmaster photometer held 30 cm away. The light source, a 100 W clear glass bulb, was also 30 cm away from the cloth. In these conditions the amount of light reflected from each cloth, in lumens per sq. ft., was black: 2, dark grey: 6, light grey: 12 and white: 30 .

A single screen (1.8 × 0.9 m) was used in experiments 1 and 5, in which the two sides were compared as regards attractiveness. In experiment 2, two screens were set up side by side (0.6 m apart) and joined together by wood so that they could be carried in one piece. In this experiment one screen was compared with the other. In experiment 3, three separate screens were used which differed from each other in size. The legs of all screens were 60 cm long.

In addition to *H. insidiatrix* the following biting flies settled on the screen: Tabanidae: *Haematopota rubens* Austen, *Atylotus fuscipes* Ricardo, *Atylotus agrestis* Wiedemann, *Tabanus taeniola-variatus* Walker, *Chrysops fuscipennis* Ricardo.

Muscidae, Stomoxydinae: *Stomoxys nigra* Macquart, *Stygeromyia maculosa* Austen, *Haematobia (Lyperosia) thirouxi* Roubaud, *Haematobia uniseriata* Malloch.

The only non-biting insect to settle on the screen in any numbers was *Oxybelus curviscutis* Arnold (Hymenoptera, Sphecidae), a predator of biting flies.

RESULTS

Experiment One

Cloths of different shades were exposed in pairs with the results shown in Table I. When black was exposed with a lighter shade, most flies settled on the black.

Flies approached and settled on the black cloth and probed its surface with their mouthparts, they then flew a short distance or walked forwards (up to 2 cm) and probed again. With a lighter cloth, flies could be seen flying within 5 cm of the surface. This was commonly observed when light grey or white was exposed. Flies settling on shades other than black seemed less likely to probe and were not so easy to catch. The numbers shown in brackets in Table I include flies which settled on the screen but were not caught.

TABLE I

Number of H. insidiatrix *caught on the two sides of a vertical cloth screen. The two sides differed only in shade: as shown. Numbers in brackets include flies which settled but were not caught.*

	Dark grey	Light grey	White
Black	(36) 31 \ 5 (10)	(39) 36 \ 4 (8)	(36) 34 \ 8 (10)
Dark Grey		(16) 11 \ 13 (18)	(31) 20 \ 18 (30)
Light Grey			(13) 7 \ 7 (9)

Flies settle on the screen when it is carried vertically, as above, and they also settle on the under-surface when the screen is carried horizontally (about 75 cm above the ground). The results with a horizontal screen are, however, quite different (Table II). No flies settled on the upper surface. The screen was

TABLE II

Number of H. insidiatrix *caught on the lower side of a horizontally held cloth screen. Each shade was compared with the others as shown. No flies settled on the upper surface, whatever its shade.*

	Dark grey	Light grey	White
Black	23 \ 27	25 \ 28	34 \ 18
Dark Grey		30 \ 31	44 \ 31
Light Grey			53 \ 18

turned over every 45 m so that the flies were collected alternately from the two shades. The transects on which Table I is based were alternated with those for Table II. More flies were caught on the lower surface of a horizontal screen than on the two sides of a vertical screen. Flies also settled on the under surface of the screen when this was of a lighter shade, or even white. These flies probed the lighter cloths as well as the dark ones and they were easily caught.

Vertical screens were used in all other experiments.

Experiment Two

In this experiment the black screen was used as a control and compared with an adjacent screen containing different amounts of white. In each case the white was added as three vertical or three horizontal stripes, spaced so as to divide the black into four equal parts. The amount of white varied between 2% and 50% of the area of the screen. The results for vertical and horizontal stripes are shown in Figure 1.

In each case the percentage of the total flies caught on the black and white screen decreased as the amount of white in this screen increased.

The regression coefficients are: vertical stripes, $b = -0.878$; horizontal stripes, $b = -0.593$, the difference between these is statistically significant, $t = 2.19$, $P = < 0.05$. The values of Y at $X = 22.0$ are also statistically different, $t = 4.04$, $P = < 0.001$. A screen with 50% white attracted very few flies whether the stripes were vertical or horizontal. Even a small amount of white reduced the attractiveness of a screen, however, particularly if these white stripes were horizontally arranged.

Experiment Three

To determine whether or not this decreased attractiveness was the result of a reduction in the area of black as more white was added, black screens of different area were compared: 24 sq.ft. (1.8 × 1.2 m), 16 sq.ft. (1.5 × 0.96 m) and 8 sq.ft. (1.2 × 0.6 m). The screens were carried in procession and their order changed after approximately 10 flies had been caught. Each person collected an equal number of times on each screen and in each position in the order of procession. More flies were caught on the first screen (100) than on the second (58) or third (60).

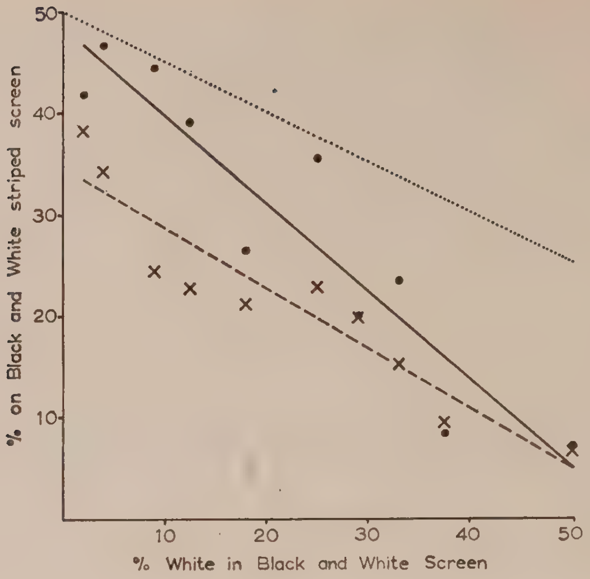


Figure 1. The percentage of the total flies that settled on the black and white side of the screen, with different amounts of white in the screen. The black side was a control in each comparison. The white was added as VERTICAL STRIPES (dots and unbroken line) and as HORIZONTAL STRIPES (crosses and broken line). The dotted line shows the values which would be expected if the smaller catch were due simply to the reduced area of black (as found in Experiment 3).

The size of the screen was also important, 99 flies being caught on the largest screen, 76 on the medium sized screen and 43 on the smallest screen. Approximately the same number of flies were caught per square foot of surface. The reduced area of black in a black and white screen is not sufficient, therefore, to explain the reduced catch found in experiment 2. The dotted line in Figure 1 shows the

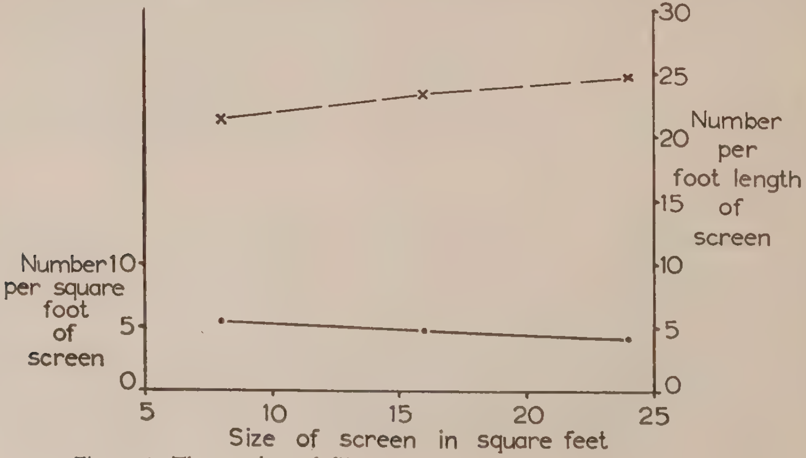


Figure 2. The number of flies caught on screens of different sizes.

values which would be expected if the smaller catch on black and white striped screens was due simply to the reduced area of black. This might however be an overcorrection since the all-black screens also decreased in length as their area was reduced. The lower catch on the smaller screens could therefore be related to either of these changes (Figure 2).

Experiment Four

It seemed that some help in interpreting experiments 1 to 3 might be derived from observations of the position of attack by *H. insidiatrix* females on a host animal. A donkey was led through the bush and collections of flies made at intervals, as with the screens. Figure 3 shows the positions in which 151 flies were caught. The position in which 50 successive flies settled on a black screen is also shown. These observations suggest that the lower part of the screen might be particularly important in determining the settling response.

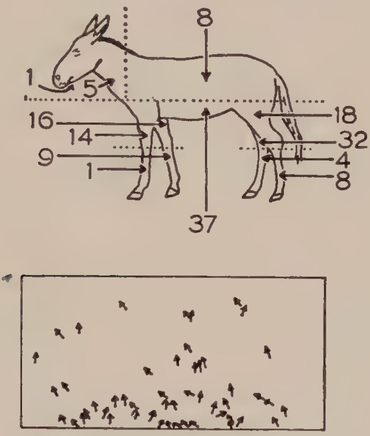


Figure 3. The number of *H. insidiatrix* caught on different regions of a donkey's body and the position in which they settled on a black cloth screen.

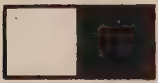
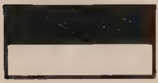
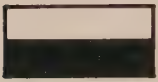
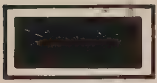
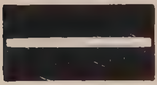
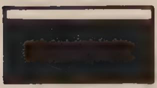
Experiment Five

To investigate the effect of the position of white in a black and white screen, each of the arrangements shown in Table III was compared with an all-black control on the other side of the screen. The experiment was carried out in December 1959 when there were few flies coming to the screen and again in January 1960 when the population was apparently higher (BARRASS, 1960b). With differently sized black screens, approximately the same number of flies were caught per square foot of surface. The differences in the numbers of flies caught on the black and white screens, when each arrangement is compared to a black control (OBSERVED RATIO in Table III), could be the result of differences in the area of black in the screens. The EXPECTED RATIO (Table III) is calculated on this assumption. The values of X^2 in the table are based on the null hypothesis that the number of flies caught is proportional to the area of black in the screen.

A 15 cm wide strip below the black or arranged horizontally in the centre of the black did not reduce the attractiveness of these screens any more than would be expected with the reduced area of black. Each of the remaining four arrangements was 50% black and 50% white. Conflicting results were obtained with white around black; in one case the number of flies caught was not statistically different from the expected 1 : 2 ratio and in the other approximately the same number of flies were caught on the two sides of the screen. With white below black fewer flies were caught on the black and white screen than expected and in one case this difference was statistically significant. With black below white the result again differed from expectation but this time more flies settled on the black and white side (per unit area of black) than on the control. In one case this difference was statistically very significant. In the final arrangement the length of the screen was divided so as to be half black and half white. Approximately half as many flies were caught on the test screen as on the control.

TABLE III

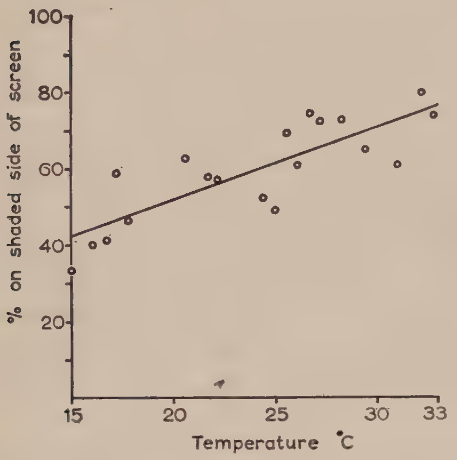
Each arrangement of black and white was compared with a black control and the number of flies caught in each comparison is shown

Arrangement of Black and White		Ratio of flies on B.W.: on control		X^2	P	Date
		Observed ratio	Expected ratio			
	1)	35 : 37	33 : 39	0.224	<0.75	8.12.59
	2)	22 : 28	23 : 27	0.080	<0.9	12. 1.60
	1)	21 : 27	22 : 26	0.084	<0.9	16.12.59
	2)	23 : 41	29 : 35	2.270	<0.25	12. 1.60
	1)	26 : 25	17 : 34	7.147	<0.01	10.12.59
	1)	48 : 80	43 : 85	0.833	<0.50	9. 1.60
	1)	22 : 33	18 : 37	1.321	<0.25	13.12.59
	2)	9 : 41	17 : 33	5.705	<0.025	9. 1.60
	1)	24 : 31	18 : 37	2.793	<0.1	14.12.59
	2)	57 : 66	41 : 82	9.366	<0.005	9. 1.60
	1)	16 : 34	17 : 33	0.088	<0.9	15.12.59
	2)	26 : 40	22 : 44	1.091	<0.5	9. 1.60

The attraction of *H. insidiatrix* to screens depends not simply on the area of black available but more particularly on the position of black in relation to the white part of the screen.

Experiment Six

Tsetse flies (*Glossina morsitans* Westw.) show a preference for the sunny side of a cloth screen (BARRASS, 1960a). Observation indicated that this was not true of *H. insidiatrix.* To determine whether or not this preference was affected by climatic conditions, transects were made once each hour throughout the day on three days. The temperature and humidity at the start of each transect were recorded and the number of flies caught on the sunny and shaded sides of the screen were counted. Measurements of air temperature and humidity were made with a whirling hygrometer held in the shade of the observer's body.



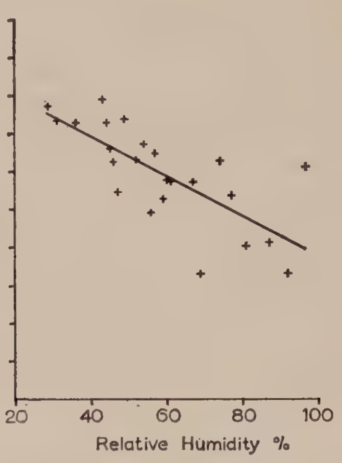


Figure 4. (Left) The percentage of the total flies that settled on the shaded side of a black cloth screen, plotted against temperature.

Figure 5. (Right) The percentage of the total flies that settled on the shaded side of a black cloth screen, plotted against relative humidity.

The results given in figures 4 and 5 indicate that the sunny side of a screen is preferred below 19° C and above 77% R.H. At higher temperatures and lower humidities this preference is reversed. Changes in temperature and humidity occurred together when these observations were made, so it is not possible to say which was the most important in effect on the change in behaviour. Regression lines for temperature and relative humidity against percentage flies on the shaded side were drawn from these data, and are shown in figures 4 and 5. The correlation between temperature and percentage flies on the shaded side is stronger ($r = 0.823$, $t = 10.506$, $P = < 0.001$) than that for relative humidity ($r = -0.719$, $t = 4.841$, $P = < 0.001$) but the difference between these correlation coefficients is not statistically significant.

DISCUSSION

Previous work on the settling of biting flies on surfaces of different colours has been reviewed briefly by BARRASS (1960a).

For *Glossina,* see MALDONADO (1910), SIMPSON (1911), SWYNNERTON (1933), LLOYD (1935) and JACK (1939); for *Culex* and *Aedes,* see KO (1925), GJULLIN (1947) and BROWN (1951); for *Simulium*, see DAVIES (1951); and for *Lyperosia,* see KRIJGSMAN & WINDRED (1931), THOMSEN (1938) and HAMMER (1941).

PORTCHINSKY (1915) records that *H. pluvialis* L. settled on black surfaces but avoided white ones. CURZON (1924) states that *Haematopota* sp. alighted on the black part on an animal's coat rather than the white part.

More *H. insidiatrix* settled on a black vertical surface than on a lighter shade. When a screen was carried so that its surface was parallel to the ground, many flies were caught on its under surface and the shade of the screen seemed to have much less effect. When a black and white striped cloth was compared to a black control screen (both held vertically), a smaller percentage of the flies settled on the black and white as the width of the white stripes was increased. Even a small amount of white reduced the catch, particularly when the stripes were horizontal. This reduced catch was not simply the result of reducing the amount of black in a screen, but with the all-black screens used in the present study the number of flies caught was proportional to the area of the screens.

When screens comprising different arrangements of black and white were compared with a black control screen of the same total area, the results could not all be explained in terms of differences in the area of black. When white was below black the catch was less and when it was above black the catch was greater, than expected.

Flies seem most likely to settle on a surface which:

1. is dark in shade, particularly in its lower parts,
2. is unbroken by white stripes,
3. has a flat horizontal lower surface, and
4. offers shade. It is only at low temperatures and high humidities that the sunny side is preferred.

The settling of *H. insidiatrix* on a screen may be interpreted as being part of the host-finding behaviour. Only the blood-sucking females are attracted and they probe the surface with their mouthparts.

JACK & WILLIAMS (1937) drew attention to the reversal from positive to negative phototaxis in *Glossina morsitans* Westwood at high temperatures. Perttunen has reviewed the literature concerning such reversals of the sign of phototaxis in other insects, in response to changes of temperature (PERTTUNEN, 1959) and to changes in humidity (PERTTUNEN & LAHERMAA, 1958). In view of these laboratory observations, the present results in the field are of interest. In the present work it is not possible to distinguish between the effects of temperature and humidity. Very significant correlations were found between changes in both of these factors and the preference of *H. insidiatrix* for the sunny or shaded side of the screen.

I wish to thank Mr. L. H. Stewart, Director of National Parks in Southern Rhodesia, for permission to work at Wankie Main Camp and Mr. E. C. Davison, Chief Game Warden, for his help in many ways and for the co-operation of his staff. Professor E. B. Edney and Dr. E. Bursell read and criticised the manuscripts for this paper and Professor B. H. Rasmussen prepared the German summary. I am also indebted to Mr. H. Oldroyd of the British Museum (Natural History) for identifying *H. insidiatrix* Austen and the other Tabanidae and Muscidae which settled on the screen, and to Dr. G. Arnold, formerly of the Bulawayo Museum, for confirming the identification of *Oxybelus curviscutis* Arnold. I was helped in the collection of insects by my assistant, Mr. G. Nyamakunda. Acknowledgement is made to the Wellcome Trust for a grant which made this work possible.

ZUSAMMENFASSUNG

DIE WAHL VON STOFFSCHIRMEN VERSCHIEDENER HELLIGKEIT DURCH WEIBLICHE HAEMATOPOTA INSIDIATRIX *AUSTEN (DIPTERA, TABANIDAE)*

Die Reaktion der weiblichen *Haematopota insidiatrix* auf Stoffschirme wird als ein Teil des Verhaltens bei der Suche nach einem Wirtstier betrachtet. Beim Vergleich von schwarzen, dunkelgrauen, hellgrauen und weissen Stoffen in Beziehung auf ihre Anziehungskraft setzten sich mehr Fliegen auf die schwarzen Stoffe als auf anders getönten. Wenn ein Schirm horizontal getragen wurde, setzten sich die Fliegen auf die untere Seite doch nicht auf die obere Seite. Ausserdem stieg die Anzahl der gefangenen Fliegen. Ein schwarzer Schirm mit drei weissen Streifen versehen übte um so weniger Anziehungskraft aus, je breiter die Streifen gemacht wurden. Selbst enge weisse Streifen verminderten die Anzahl der gefangenen Fliegen, besonders wenn sie horizontal angebracht waren. Wenn Schirme von verschiedener Grösse herumgetragen wurden, fingen sich auf den kleineren Schirmen weniger Fliegen. Die Fliegen setzten sich auf den unteren Teil der Schirme genau wie sie es bei Wirtstieren machen. Wurde ein Schirm in eine untere Hälfte (weiss) und eine obere Hälfte (schwarz) geteilt, so liessen sich weniger Fliegen darauf nieder als auf der ganzschwarze Rückseite (Kontrolle). Im umgekehrten Falle, (untere Hälfte schwarz, obere Hälfte weiss) wurde ungefähr dieselbe Anzahl von Fliegen auf dieser Seite wie auf der völlig schwarzen Rückseite gefangen. Bei Temperaturen unter 19° C und einen relativen Feuchtigkeit von über 77% setzten sich mehr Fliegen auf die sonnige Seite als auf die beschattete Seite des Schirmes. Bei höheren Temperaturen und niedrigerem Luftfeuchtigkeitsgehalt war diese Vorliebe umgekehrt.

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LABORATORY CULTURE OF *AEDES (FINLAYA) TOGOI* (THEOBALD), 1907 AND MEASUREMENTS OF ITS SUSCEPTIBILITY TO INSECTICIDES

BY

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The literature on *Aedes togoi* is reviewed. This oriental mosquito occasionally acts as a vector for filarial or virus diseases and is sometimes a nuisance from its bites. Eggs from Taiwan (Formosa) were brought to London in 1958. A laboratory colony was reared from these eggs and maintained by methods similar to those commonly used for *Aedes aegypti*. Various aspects of the bionomics were studied, including the lengths of stages, mortality during development and the effects of copulation, feeding and salt content of water on oviposition. The levels of susceptibility to DDT, BHC and dieldrin were measured by the World Health Organisation (W.H.O.) method and compared with those of colonies of *A. aegypti* originating in Taiwan and the Pescadores.

Aedes togoi was first described by THEOBALD (1907) under the name *Culicelsa togoi*. The description was based on two females caught in Osaka, Japan. Since then a considerable number of papers has appeared dealing with its distribution, systematics, bionomics, and medical and veterinary importance. However, so far as is known, no record of its laboratory colonization is available and consequently its biology is still largely unknown. Successful laboratory colonization has enabled me to make preliminary observations on its biology and measurements of its susceptibility to insecticides.

In the course of this study I examined a large number of papers dealing with this species, and a brief review of the literature is given. No attempt is made to discuss the taxonomy of *Aedes togoi*. The records of its distribution are not given in detail so far as localities are concerned, but indicated with countries or states as a whole.

The distribution of *Aedes togoi* has been recorded from eastern Siberia, Korea, China, Hong Kong, Japan, Ryukyu Islands, Bonin Islands and Volcano Islands. The immature stages have been reported to breed in artificial containers containing fresh water near human habitations in Japan (YAMAGUTI & LACASSE, 1950), Manchuria and Korea (PETRISHCHEVA, 1948), and in brackish rock pools just above high tide in China (FENG, 1938; HISAO & BOHART, 1946), some parts of Japan (HSIAO & BOHART, 1946; YAMAGUTI & LACASSE, 1950; OMORI & FUJII, 1953) and eastern Siberia (PETRISHCHEVA, 1948). According to JACKSON (1938), in Hong Kong the larvae had been found in waters of salinities ranging from 0.046 to 1.85 per cent chlorine (as chlorides in his original paper), the highest percentage being approximately that contained in sea water.

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Adult females of this species have been experimentally demonstrated to be readily infected with *Wuchereria bancrofti* (MOCHIZUKI, 1913; YAMADA, 1927; JACKSON, 1936), *Setaria digitata* (ITAGAKI, TANIGUCHI & KAWATA, 1946 & 1947), Japanese B encephalitis virus (MITAMURA, KITAOKA, WATANABE, TENGIN, HOSOI, SEKI, NAGAHATA, JO & SHIMIZU, 1939; HAMMON, TIGERTT, SATHER & SCHENKER, 1949) and Semliki Forest virus (NYE & LIEN, 1960). OSHIMA (1956) reported a low incidence (0.94%) of natural infection of filarid larvae in this species in epidemic areas of *W. bancrofti* in Western Kyushyu, Japan. PETRISHCHEVA (1948) claimed that this species together with *Aedes japonicus* were chief vectors of Japanese B encephalitis in the Soviet Far East. However, as a vector of human disease in nature its status is doubtful (BOHART, 1956).

According to FENG (1933, 1935) and OMORI & FUJII (1953) adults are rarely seen in houses; nevertheless JACKSON (1938) states that adult females constitute a notorious mosquito nuisance. OMORI & FUJII (1953) were able to collect 2,681 females in six monthly collections using human-baited traps operated hourly throughout the night and sometimes part of the day. They found that females appeared to feed at any time throughout the 24 hours, the main peak being 1—3 hours after sunset, the second one just after sunrise. CHANG, SUN & WU (1957) conducted routine collections in one each of various animal shelters and reported that the percentages caught were: 49.6 (194) from a horse shelter, 25.8 (101) from a cowshed, 23.5 (92) from a fowl-run, and 1 (4) from a human dwelling. OMORI & FUJII (1953) pointed out that this species prefers cattle to human beings and when cattle are not available, the females readily bite human beings. PETRISHCHEVA (1948) observed that this species occurred over a vast coastal area where blood sources seemed to be unavailable. OMORI & FUJII (1953) observed that even when immature stages occurred in large numbers, only a small proportion of resulting females was attracted to various animals; the rest remained undiscovered.

OMORI & FUJII (1953) mentioned that in human-baited trap collections they were able to collect only three males despite the presence of many breeding places around the collection site. They consider that copulation probably takes place under peculiar conditions, i.e. with respect to place and time.

Observations on seasonal occurrence were conducted by several workers in Japan (MITAMURA, KITAOKA & IMAI, 1950; OMORI & FUJII, 1953) and the Soviet Far East (PETRISHCHEVA, 1948). Although the results are variable, it is generally agreed that adults were found between May and November, the peak being in summer months, June to September. PETRISHCHEVA (1948) and ISHII, NAKAYAMA & ISHII (1954) reported that hibernation occurred only in the egg stage.

PETRISHCHEVA (1948) considered that *Aedes togoi* and *Aedes japonicus* are changing their original mode of life to a synanthropic one, according to her field observations in the Soviet Far East, Manchuria and Korea. A summary of her observations taken from *Rev. Appl. Ent.*, B, 1949, 146, is briefly as follows: In the southern part of Maritime Province of the Soviet Far East both species are typically associated within the regions uninhabited by man, *Aedes togoi* being restricted to the rocky coast and *Aedes japonicus* to the primeval forest, while in northern Korea and Southern Manchuria both species had lost all connection with their original habitats and are abundant and completely domesticated in densely populated towns and villages. They become adapted to an association with men as soon as the opportunity arises. The first association of *Aedes togoi* with men occurred in fishing camps, quarries and small settlements connected with light houses, especially those that harboured some cattle or horses. Therefore, he considered that similar spread may occur in the Soviet Far East as the settlement of uncultivated areas progresses, and warned that prophylactic measures should be taken in new settlements in previously unpopulated regions, particularly in natural foci of Japanese B encephalitis.

Ishii *et al.* (1954) studied the effect of temperature on growth in *Aedes togoi* and *Aedes albopictus.* Their findings for *Aedes togoi* are summarized as follows: The eggs kept at 10° C and 15° C for 15 days did not hatch at all, but as soon as the temperature was raised to 20° C they hatched on the 4th day. At 25° C they hatched in 2—3 days and at 35° C they hatched but died before pupation. The groups of larvae kept at 10°, 15°, 20°, 25°, 30° and 35° C were observed for 16 days or less. Below 20° C none of them pupated; at higher temperatures, 20%, 10% and 0% succeeded in emerging at 25°, 30° and 35° C respectively (at 35° C 100% died within 10 days). Among those kept at 25° C and 20° C pupation took place 5—6 and 6 days respectively after hatching. The mortalities of the adults kept at 10° C, 30° C and 35° C within 10 days were 70%, 60% and 70% respectively. The mortality of adults kept between 15° C and 25° C was lower than those maintained at other temperatures. They also made observations on the proportion of sexes resulting from the batches of larvae hatched in each calendar month from June to October. They mentioned that both sexes were almost equal in proportion. However, the monthly male ratios, calculated by the writer based on their results, were 1.06, 0.93, 0.88, 0.93 and 2.05 for June, July, August, September and October respectively. The number of males resulting from the October batch was double the number of females. This may indicate a possible correlation between the sex ratio and seasonal fluctuation of temperature since, in the Northern Hemisphere, July and August (with the low male ratio) are generally hotter than June, September and October (with high proportion of males).

LABORATORY CULTURE OF AEDES TOGOI

Source of the material

In October, 1958 I brought a batch of about 2,000 eggs of *Aedes togoi* from Taiwan and started the laboratory culture in the Department of Entomology, London School of Hygiene and Tropical Medicine. Those eggs were laid on 12 September 1958 by the females of the laboratory colony of *Aedes togoi* in the Department of Entomology, U.S. Naval Medical Research Unit No. 2 (NAMRU-2), Taipei, Taiwan, where the adult mosquitoes were liberated and fed on rabbits in a large temperature-controlled and well humidified insectary. The insectary was provided with several large wooden larval breeding tanks, lined with brown crepe papers for oviposition, and the water in the tanks was kept aerated to prevent formation of scum. The original stock was established by Dr. Stephen M. K. Hu from a large number of larvae and pupae collected from brackish pools along the rocky coast near Chinshan, Taipei Hsien, Taiwan.

Insectary conditions

Except where otherwise indicated, routine maintenance and biological observations of the culture were undertaken in an unhumidified insectary at a temperature of 27 ± 3° C. As the insectary is situated on the roof of the School building it receives natural light fairly well during the day. Artificial light was used during the dark hours.

Routine maintenance of adults

Adult mosquitoes were confined in an elongate cage measuring 25 × 25 × 50 cm. As described by LAURENCE & SMITH (1958), the cage is made of wood and plastic mosquito netting, with a net sleeve. Since the insectary was not humidified, two large pieces of damp lint were used to cover the cage. Sugar solution on a cotton wool pad was provided and renewed every 3 or 4 days. For blood meals an anaesthetized guinea-pig was placed in the cage for a couple of hours once a week and after each blood meal an enamelled bowl 7 cm deep and 14 cm in diameter containing water and lined with Whatman No. 1 filter paper was placed in the cage for oviposition.

Routine maintenance of young stages

Newly oviposited eggs were left moistened in the oviposition bowl in the cage for a few days and after removal from the bowl, egg papers were kept in a well saturated glass jar for several days and were then dried for storage. For breeding of larvae, polythene bowls 15 cm deep and 30 cm in diameter were used. After the eggs were immersed in water, a small pinch of yeast-Bemax* mixture was added. The larvae usually hatch within 24 hours after immersion. A small pinch of yeast-Bemax mixture was added daily, and the breeding water was kept aerated to prevent formation of scum. After pupation, the pupae were picked up by a pipette and transferred into a small enamelled bowl. The bowl was then placed into the adult cage for emergence. This procedure was repeated once every two weeks.

Incubation and hatching

An experiment was made to find the period required for the embryo to complete its development within the egg. Newly laid eggs were held in a well saturated glass jar. At daily intervals, over a period of 6 days, batches of eggs were immersed in water. The test eggs were counted prior to immersion in water and the hatched larvae were counted 24 hours after immersion. The result revealed that early drying or immersion of eggs causes little or no hatch. The minimum period required for incubation in the majority was found to be 4 days. A batch of eggs which had been kept moistened for 6 days and stored dry for 15 days also hatched quite satisfactorily. The result is tabulated in Table I.

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* Bemax is a preparation from stabilized wheat germ which contains vitamin B complex, protein and mineral supplement.

TABLE I

Incubation and hatching

Number of days for which the eggs had been kept moistened	Percentage hatch within 24 hours of immersion in water at indicated number of days after oviposition (Numbers are shown in parentheses)							
	0	1	2	3	4	5	6	21
0	0 (0/559)	—	0 (0/200)	0 (0/200)	0 (0/200)	0 (0/200)	0 (0/200)	—
1	—	0 (0/451)	—	—	—	—	—	—
2	—	—	0* (0/410)	—	—	—	—	—
3	—	—	—	0.6 (2/334)	—	—	—	—
4	—	—	—	—	96.7 (563/580)	—	—	—
5	—	—	—	—	—	92.3 (406/440)	—	—
6	—	—	—	—	—	—	95.2 (750/755)	8.15 (88/108)

* In this batch 1 each hatched respectively at 5, 7, 8 days after immersion in water.

It was found, in this species, that after completion of embryonic development within the eggshell the larvae do not break out if the eggs are maintained under moist but not submerged conditions. They usually do not break the shells even when they are kept moistened for a period of up to two weeks. However, it is quite different in *Aedes aegypti.* In a moist atmosphere the larvae of *Aedes aegypti* usually break their eggshells as soon as their embryonic development is completed, and force their way out.

In many instances, the hatching of larvae from batches of eggs stored in dry condition for more than a month or so was found to be unsatisfactory. Such a period of storage in dry conditions causes little or no hatch. Therefore, it is advisable to use fresh eggs soon after they have been kept moistened for 4 or 5 days.

Duration of aquatic stages

An observation was made of 400 individuals. Within an hour of hatching 400 newly hatched larvae were divided into two groups, each consisting of 200. They were kept in two glass jars 15 cm deep and 25 cm in diameter, and sufficient food was supplied every day. The first moult was observed at two days, the second moult at 4 days, the third at 7 days after hatching from eggs. When the third moult was observed there were many larvae in the third instar (84%). The formation of pupae occurred at 10 days and continued until 16 days after hatching from eggs. The emergence of adults took place 12—19 days after hatching from eggs. The speed of growth, survival rate and sex ratio are tabulated in Table II, and the pattern of pupation and emergence in Table III.

TABLE II

Speed of growth, survival rate and sex ratio among 400 individuals observed

No. of days after hatching from eggs	Stages	Survival Rate (%)	Sex Ratio (%) Male	Sex Ratio (%) Female	Remarks
0	1st instar	100(400)	—	—	Numbers are shown in parentheses.
2	2nd instar	—	—	—	
4	3rd instar	—	—	—	
7	4th instar	91(363)	—	—	3rd instar 84(337) 4th instar 7(26)
10—16	Pupa	86(345)	47(161)	53(183)	
12—19	Adult	83(332)	48(159)	52(173)	

These results are quite different from those of Ishii *et al.*, who observed pupation 5—6 and 6 days after hatching at 25° C and 20° C respectively; whereas in this strain pupation took place 10—16 days after hatching, at 27 ± 3° C.

TABLE III

Percentage pupation and emergence

No. of days after hatching from eggs	% Pupation Male	% Pupation Female	% Emergence Male	% Emergence Female	Remarks
10	28.4(46)				Numbers are shown in parentheses
11	46.9(76)	11.5(21)			
12	19.8(32)	35.0(64)	7.5(12)		
13	4.3(7)	31.1(57)	37.7(60)	0.6(1)	
14	0 (0)	12.6(23)	39.0(62)	16.8(29)	
15	0.6(7)	7.1(13)	13.2(21)	34.7(60)	
16		2.7(5)	1.9(3)	31.2(54)	
17			0 (0)	9.8(17)	
18			0.6(1)	6.4(11)	
19				0.6(1)	

Mating in cages

After emergence of the adults of the first generation of the laboratory culture in London, the first question I encountered was whether or not *Aedes togoi* mate in captivity in cages. I tried to confirm mating activity in the cage visually, but failed. However, later they laid viable eggs. Whereas *Aedes aegypti* can be seen mating actively in small cages, the mating of *Aedes togoi* in the cages has not been witnessed by the writer (during daytime only) even after routine maintenance of the culture for more than 8 months. The dissection of females confirmed that they mate very well in all types of cages used, the smallest being 19 × 19 × 19 cm. The results are tabulated in Table IV. Although the number dissected is not large enough to give a very precise picture of mating activity in cages, the results seem to suggest that mating does not take place within a couple of days or more after emergence at least in the condition of captivity.

TABLE IV

Mating in cages

Type of cages	Number of mosquitoes at beginning Female: Male	Number of days after emergence when dissected	Percentage of females with spermatozoa in spermatheca	Remarks
A	>500 : >500	15	100(5/5)	A = 25×25×50 cm
B	146 : 74	0	0(0/25)	B = 25×25×25 cm
B	146 : 74	1	0(0/25)	
B	146 : 74	4	12(3/25)	
B	146 : 74	28	100(4/4)	
B	274 : 290	17	100(20/20)	
B	274 : 290	43	100(10/10)	
B	274 : 290	52	100(8/8)	Separated from males 17 days after emergence
C	26 : 7	1	0(0/26)	C = 19×19×19 cm
C	56 : 11	7	32(8/25)	
C	56 : 11	15	60(15/25)	
C	27 : 13	15	90(19/21)	
C	15 : 58	16	100(3/3)	
C	35 : 31	17	96(23/24)	
C	9 : 6	32	100(3/3)	

Ovarian development and autogeny

On dissection of three groups of adult females which had been kept fed on sugar solution together with males for 16—17 days, an interesting phenomenon was observed. Although no blood meal or any other food containing protein was given to those mosquitoes, some of the females were found with developing follicles. The stages of follicular development mentioned below are based on the definition by MER (1932). Since there are no floats of eggs of this species, appearance of floats at stage V is not considered. Among 55 females dissected, 14 were found with follicles at stage V, while others were found with follicles at stages I—II, II, or III. None of them were found with follicles at stages N, I, or IV. The total number of follicles at stage V in each mosquito ranges from 10 to 73 (10, 14, 17, 27, 27, 37, 39, 40, 42, 45, 50, 52, 59, 73). This is obviously a sign of autogeny in this mosquito. The percentages of females with developing follicles are shown in Table V.

TABLE V

Percentages of females with follicles at different stages

Number of days after emergence	Percentages of females with follicles at indicated stages							
	Rudimentary	N	I	I-II	II	III	IV	V
17	0(0)	0(0)	0(0)	10(2)	35(7)	5(1)	0(0)	50(10)
17	4(1)	0(0)	0(0)	28(7)	48(12)	4(1)	0(0)	12(3)
16	0(0)	0(0)	0(0)	36(4)	45(5)	9(1)	0(0)	9(1)

Numbers are shown in parentheses.

The autogeny was further confirmed by the fact that four groups of females which had been fed only on sugar solution laid eggs after 7 or more days after emergence. The results are shown in Table VI. The autogenous eggs laid by mated females were found viable and developed normally into adults.

TABLE VI

Autogenous oviposition

Group	Cages used	No. of mosquitoes contained in the cages	Number of eggs laid (*days after emergence)									
		Female: Male	*1—6	7	8	9	10	11	12	13	14
I	C	53 : 15	0	53	2	38	(—	—	81)	46	0
II	C	112 : 331	0	17	161	(—	—	301)	365	44	61
III	C	32 : 35	0	28	0	0	20	(—	—	22)	0
IV	C	58 : 89	0	0	11	0	7	(—	—	38)	0

Group	*15	16	17	18	10	20
I	15	(—	—	7)	0	19
II	215	0	0	0	0	49
III	14	0	0	0	0	0
IV	0	0	0	0	0	0

Oviposition after taking a blood meal

It is obvious from the above results that autogenous oviposition takes place 7 days or more after emergence. However, the detection of normal oviposition after blood meals seems to be greatly complicated by the autogenous development of ovaries.

Two groups of mosquitoes, 33 and 41 females, which had fed only on sugar solution for 15 days and then fed on blood of baby mice, suddenly laid a small number of eggs on the following day and continued laying eggs for 23 days, the oviposition being observed almost every day except a few days of interruption.

In another group of 50 females which had been given a human blood meal when 17 days old, oviposition took place 11 days after the blood meal and continued for 13 days. The oviposition was again noted 35 days after the blood meal. This was perhaps due to autogeny.

Observations were also made on 4 more groups. However, the results are quite variable. The results are summarized in Table VII.

Although no comparable figures can be given as to its blood preference, it was felt that females of this species bite a human hand more readily than an anaesthetized guinea-pig. However, in most cases, after offering an anaesthetized guinea-pig for a couple of hours, most of the females were found engorged. Females confined in small tubes 8 cm high and 5 cm in diameter were reluctant to feed on baby mice.

TABLE VII

Oviposition after taking a blood meal

Group	No. of females	Mating	Type of blood meal	Age of females when fed	Number of eggs laid (*days after the blood meal)									
					*1	2	3	4	5	6	7	8	9	10
I	31	Yes	Mouse	15 days	18	4	45	121	(—	89)	0	25	53	174
II	41	Yes	Mouse	15 days	8	28	77	108	(—	213)	198	176	91	88
III	50	Yes	Man	17 days	0	0	0	0	0	0	0	0	0	0
IV	18	No	Man	6 days	0	0	0	0	188	10	7	0	(—	17)
V	12	Yes	Man	6 days	0	0	0	0	0	0	0	0	0	0
VI	21	No	Gu.-Pig	6 days	0	0	0	0	0	0	0	0	0	(—
VII	27	Yes	Gu.-Pig	6 days	0	0	0	0	0	0	0	0	0	(—

Group	11	12	13	14	15	16	17	18	19	20	21	22	23	24
I	149	(—	0)	73	92	56	(—	38)	(—	—	22)	7	15	0
II	82	(—	131)	78	87	96	(—	117)	0	0	0	7	0	0
III	16	17	16	260	(—	171)	(—	—	150)	164	223	105	0	0
IV	(—	—	132)	191	141	341	(—	—	142)	428	0	0	0	0
V	0	0	0	0	0	0	(—	—	191)	211	190	0	0	0
VI	—	464)	214	106	100	(—	—	81)	84	150	151	25	(—	—
VII	—	145)	198	355	143	(—	—	146)	336	136	86	58	(—	—

Group	25	26	27	28	29	30	31	32	33	34	35	36—40	41	42
I	0	0	0	0	0	0	killed							
II	0	0	0	0	0	0	0	0	0	0	0	0	0	killed
III	0	0	0	0	0	0	0	0	97	0	0	0	10	killed
IV	0	18	0	killed										
V	0	0	0	killed										
VI	6)	92	52	0	0	(—	—	84)	3	0	killed			
VII	31)	77	53	0	0	0	0	0	18	15	killed			

Effect of copulation on oviposition

In order to confirm whether or not females do lay eggs without previous and subsequent copulation with males, two series of experiments were carried out. For the first series, two groups of mosquitoes were set up. The first group was chosen by picking up the pupae of larger size, as the females of this species in the same culture are distinctly bigger than the males, and the second group by collecting the pupae of any size. The two groups of pupae were then confined in separate cages measuring 25 × 25 × 25 cm. To make sure that all the adults emerging from the first group were females, a careful examination was made and all were found to be females only. The females of both groups, when 6 days old, were fed simultaneously on human blood. After the blood meal, the fully engorged females were transferred into smaller cages measuring 19 × 19 × 19 cm. The first group consisted of 18 females, the second group 12 females with 24 males provided for copulation. In the first group (without any contact with males) oviposition took place 5 days after the blood meal and continued for 16 days, while in the second group (with constant contact with males) oviposition did not take place until the period between 17th and 19th days after the blood meal, and oviposition continued for a few days or so.

In another series, two more groups were likewise set up and fed on guinea-pigs when 6 day old. They were confined in cages measuring 25 × 25 × 25 cm. Oviposition took place in both groups during the period between the 9th and 11th days after the blood meal and continued until the 27th day after the blood meal. The results of both series were shown in Table VII under group categories IV, V, VI and VII. From this it is quite clear that unmated females lay eggs as constantly as mated females. The eggs laid by unmated females were not viable.

Selection of oviposition site

In order to see how the adult females select oviposition sites, the following experiment was undertaken. Four glass tubes 5.5 cm high and 3.5 cm in diameter lined with 4 cm wide filter papers and containing 25 ml of tap water, 2% NaCl, 5% NaCl, 16% sugar solutions, respectively, were used. They were placed in the culture stock cage to form a 15 cm square block, and were left for 3 nights. All other possible oviposition sites were excluded from the cage. The result revealed that females avoid laying eggs in those tubes containing NaCl solution. NaCl used in this experiment was fairly pure (Sodium Chloride, May & Baker Ltd., Dagenham, England) and the sugar used was ordinary cubed table sugar. The results are shown in Table VIII.

TABLE VIII

Selection of oviposition site

Tube	Type of site	Number and percentages of eggs laid
I	Tap water	2268 (85.4%)
II	2% NaCl solution	0 (0%)
III	5% NaCl solution	9 (0.3%)
IV	16% sugar solution	369 (14.3%)

Effect of NaCl concentrations on hatching of larvae and their growth

In nature this strain of mosquito breeds in brackish rock pools near the coast. However the laboratory culture can be maintained with London tap water without adding salt. To determine the effect of NaCl concentrations on the hatching of larvae and their growth, an experiment was carried out. Five concentrations of NaCl were prepared with London tap water (pH about 8). Six 500 ml capacity beakers were used to contain 100 ml solution of each concentration, in each of which 200 eggs were immersed. The number of hatched larvae were counted 24 and 48 hours after immersion. Hatching took place at all concentrations. A higher percentage hatch was observed in 2% solution within 24 hours of immersion. On counting the hatched larvae after 24 hours of immersion, it was noted that the colour of the hatched larvae showed a slight difference according to the gradation of the concentrations. The higher the concentration, the smaller the size and the paler the colour. It was noted by the fact that it was more and more difficult to see the larvae well as the counting proceeded towards higher concentrations. This indicates that hatching is retarded by higher concentrations. After 48 hours of immersion the larvae were counted again and it was found that hatching continued in the last three higher concentrations. All the egg papers were removed then and a small pinch of yeast-Bemax mixture was given to each batch. The percentage mortality among those larvae was counted 6 and 12 days after immersion of eggs. The result is tabulated in Table IX.

TABLE IX

Effect of NaCl concentrations on hatching of larvae and their growth

	Young Stages	NaCl concentrations (gm/100 ml)					
		0	1	2	3	4	5
% & No. hatch after 24 hours of immersion	1	57%(114)	66%(132)	73%(146)	58%(116)	59%(118)	39%(77)
% & No. hatch after 48 hours of immersion	1	57%(114)	66%(132)	73%(146)	72%(143)	70%(140)	62%(123)
% & No. surviving among the hatched larvae 6 days after immersion of eggs	1	0%(0)	0%(0)	0%(0)	1%(2)	3%(4)	5%(6)
	2	0%(0)	2%(2)	5%(7)	21%(30)	54%(75)	61%(75)
	3	55%(63)	84%(111)	88%(128)	76%(108)	40%(56)	16%(20)
	4	45%(51)	5%(6)	3%(5)	0%(0)	0%(0)	0%(0)
	all	100%(114)	90%(119)	96%(140)	98%(140)	96%(135)	82%(101)
% & No. surviving among the hatched larvae 12 days after immersion of eggs	1	0%(0)	0%(0)	0%(0)	0%(0)	0%(0)	0%(0)
	2	0%(0)	1%(1)	0%(0)	2%(3)	4%(5)	3%(4)
	3	3%(3)	6%(8)	23%(33)	25%(37)	45%(63)	35%(43)
	4	96%(110)	77%(103)	72%(105)	67%(98)	43%(60)	36%(44)
	P	1%(1)	0%(0)	0%(0)	0%(0)	0%(0)	0%(0)
	all	100%(114)	85%(112)	95%(138)	95%(138)	91%(128)	74%(91)

After observation of 12 days, owing to my absence, the test larvae were not looked after and no observation was continued. Therefore, no comparable figures can be given. However, the larvae surviving from starvation for about ten days were later (discarded after 34 days from the start of the test) able to develop into adults in appreciable numbers at all concentrations. As shown in Table IX, the speed of growth is retarded at all higher concentrations throughout and the mortality is slightly higher at higher concentrations.

Tolerance of larvae to NaCl concentrations

In order to detect to what extent the larvae can tolerate NaCl concentrations, the following experiment was conducted. Ten concentrations of NaCl were prepared with London tap water (pH about 8). For the experiment 500 ml capacity beakers were used. To avoid dilution of the prepared concentrations, the larvae were first contained in small glass tubes, the water in those tubes being later withdrawn by a capillary pipette. The larvae were then washed down with the NaCl solution into a beaker containing 100 ml NaCl solution. At each concentration 25 4th instar larvae were used. They were left exposed for 24, 48, 72 and 96 hours without any food. To avoid a rapid change in concentrations by evaporation, a fourfold piece of damp lint was used to cover the beakers. As shown in Table X, the results seem to suggest that the larvae can survive from concentrations lower than 5%.

TABLE X

Tolerance of larvae to NaCl concentrations

No. of hours after exposure	Percentage mortality (concentrations of NaCl, gm/100 ml.)										
	0	1	2	3	4	5	6	7	8	9	10
24	0	0	0	0	0	0	8	76	80	84	92
48	0	0	0	0	0	0	16	80	92	92	92
72	0	0	0	0	0	0	24	80	92	96	100
96	0	0	4	8	0	8	28	84	96	96	100

MEASUREMENTS OF SUSCEPTIBILITY TO INSECTICIDES

The susceptibility of *Aedes togoi* to the insecticides commonly used in anti-malarial campaigns is practically unknown. In order to ascertain its susceptibility levels to some important insecticides, a preliminary investigation was conducted. For comparison, susceptibility tests were also conducted with two strains of *Aedes aegypti.*

Source of test insects: All the mosquitoes used in the present investigation were from the three laboratory colonies in the Department of Entomology, London School of Hygiene and Tropical Medicine. The colonies were established from aquatic stages collected from natural breeding places in 1958, *Aedes togoi* being from brackish rock pools near the coast and *Aedes aegypti* from artificial containers around houses. The localities of origin are Chin-shan, Taipei Hsien for *Aedes togoi* (Taiwan), Chi-hou, Kaohsiung City for *Aedes aegypti* (Taiwan), and Ma-kun, Pen-hu Hsien for *Aedes aegypti* (Pescadores). When the colonies were established only the first locality had been covered by anti-malarial residual DDT house-spraying since 1953.

Test methods: All the tests were carried out with the World Health Organisation standard test kits using the W.H.O. recommended procedure (W.H.O., 1958). During the tests the room was kept at 25° C and at a relative humidity of about 95%. Newly emerged adults of both sexes had been fed on 10% sugar solution for a week prior to the tests. They were given 1 hour exposure and the mortality count was done 24 hours after they were transferred into uncontaminated clean tubes. For larval tests, late 3rd instar and early 4th instar larvae were used. They were left exposed in given concentrations for 24 hours and then mortality was noted.

Results: The results of the tests are given in Tables XI and XII, and shown as log.-concentration/probit regression lines in Figures 1 and 2. As no strain of *Aedes togoi* is available from localities free from DDT contamination, the evaluation of its possible resistance to DDT cannot be done. However, it was found as susceptible as normal strains of *Aedes aegypti* from Taiwan and the Pescadores. The male adults of *Aedes togoi* are much more susceptible to DDT when compared with the females and with both sexes of normal strains of *Aedes aegypti.* In the larval stage, *Aedes togoi* is much more tolerant of DDT when compared with normal strains of *Aedes aegypti.*

TABLE XI

Susceptibility Tests on Adults

Sex	Dosage (% DDT)	Percentage Mortality		
		Ae. togoi (Taiwan)	*Ae. aegypti* (Taiwan)	*Ae. aegypti* (Pescadores)
Male	Control	0 (41)	0 (214)	0 (63)
	0.25	—	0 (204)	0 (65)
	0.5	7.4 (27)	1 (198)	0 (62)
	1	66.7 (48)	12.8 (180)	6.7 (60)
	2	82.9 (35)	84.3 (185)	83.6 (73)
	4	100 (30)	100 (161)	100 (64)
LC50		0.916	1.426	1.520
95% fiducial intervals		0.780	1.285	1.394
		1.076	1.581	1.658
Female	Control	0 (19)	0 (79)	0 (47)
	0.25	—	0 (103)	0 (45)
	0.5	—	0.7 (149)	0 (45)
	1	9.1 (22)	7.9 (114)	2.3 (44)
	2	50 (18)	60.3 (146)	43.3 (60)
	4	94.4 (18)	99.3 (145)	97.5 (40)
LC50		1.942	1.750	2.088
95% fiducial intervals		1.592	1.641	1.873
		2.370	1.866	2.330

The number of adults tested is shown in parentheses.

TABLE XII

Susceptibility Tests on Larvae

Insecticide	Dosage (ppm)	Percentage Mortality		
		Ae. togoi (Taiwan)	*Ae. aegypti* (Taiwan)	*Ae. aegypti* (Pescadores)
Control		0 (100)	0 (50)	0 (55)
DDT	0.004	—	0 (50)	27.1 (70)
	0.02	2 (100)	46 (50)	64.3 (70)
	0.1	51 (100)	96 (50)	98.6 (70)
	0.5	94 (100)	—	—
LC50		0.1071	0.0232	0.0099
95% fiducial intervals		0.0895	0.0186	0.0075
		0.1281	0.0290	0.0132
γ-BHC	0.004	0 (100)	0 (50)	0 (60)
	0.02	59 (100)	16 (50)	51.7 (60)
	0.1	96 (100)	90 (50)	93.3 (60)
LC50		0.0193	0.0388	0.0227
95% fiducial intervals		0.0125	0.0322	0.0182
		0.0297	0.0467	0.0284
Dieldrin	0.004	5 (100)	6 (50)	20 (25)
	0.02	89 (100)	84 (50)	94.3 (35)
	0.1	100 (100)	100 (50)	100 (25)
LC50		0.0097	0.0106	0.0070
95% fiducial intervals		0.0084	0.0086	0.0053
		0.0111	0.0131	0.0093

The number of larvae tested is shown in parentheses.

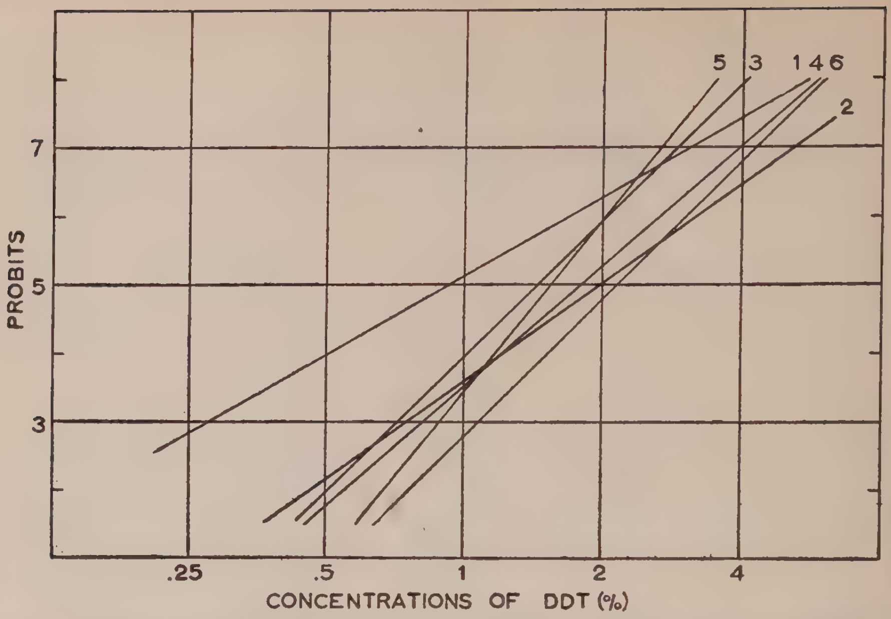

Fig. 1. Log.-concentration/probit regression lines showing the response of adult mosquitoes to DDT. The regression lines are marked with the following numerals:

	Males	Females
Aedes togoi (Taiwan)	1	2
Aedes aegypti (Taiwan)	3	4
Aedes aegypti (Pescadores)	5	6

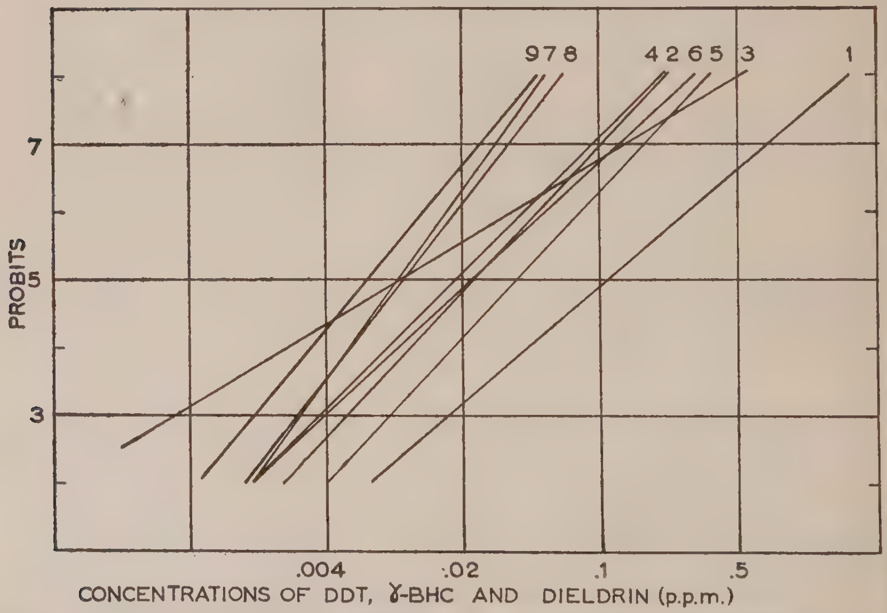

Fig. 2. Log.-concentration/probit regression lines showing the response of mosquito larvae to DDT, γ-BHC and Dieldrin. The regression lines are marked with the following numerals:

	DDT	γ -BHC	Dieldrin
Aedes togoi (Taiwan)	1	4	7
Aedes aegypti (Taiwan)	2	5	8
Aedes aegypti (Pescadores)	3	6	9

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ZUSAMMENFASSUNG

LABORKULTUR VON AEDES (FINLAYA) TOGOI *(THEOBALD), 1907, UND MESSUNGEN IHRER INSEKTIZIDEMPFINDLICHKEIT*

Eine Laborzucht von *Aedes togoi* wurde erfolgreich eingerichtet und die Kulturtechnik beschrieben. Die Ergebnisse der vorläufigen Beobachtungen über ihre Biologie werden ebenfalls mitgeteilt. Die Kulturtechnik unterscheidet sich nicht sehr von den gewöhnlich für *Aedes aegypti* angewandten. Jedoch wurde die Dauer eines Generationszyklus viel länger als die von *Aedes aegypti* befunden. Bei einem Teil der Weibchenpopulation konnte Autogenie beobachtet werden. Kopulationen wurden nicht in allen benutzten Käfigtypen durch Augenbeobachtung festgestellt, durch Sektion der Weibchen jedoch in allen Käfigformen bis zu den kleinsten von 19 × 19 × 19 cm nachgewiesen. Unbegattete Weibchen legen nachweislich ebenso gleichmäßig Eier ab wie begattete, aber die produzierten Eier waren alle steril. Die von befruchteten autogenen Weibchen abgelegten Eier waren lebensfähig und entwickelten sich zu normalen Imagines. Erwachsene Weibchen, denen 2- und 5%ige Salzlösungen, 16%ige Zuckerlösung und frisches Leitungswasser geboten wurden, vermieden Eiablagen in die Gläser mit Salzlösungen, obwohl die Larven der Stammzucht in brackigen Felsentümpeln lebend gefunden wurden. In Hongkong wurden die Larven dieser Art in Gewässern mit einer Salinität von 0,046 bis 1,85% Chlor gefunden, annähernd dem höchsten Prozentgehalt in Seewasser. In der vorliegenden Untersuchung ertrugen die Larven Salzlösungen bis zu 5% und die Eier entwickelten sich ganz normal zu Erwachsenen in Salzlösungen von 1, 2, 3, 4 und 5%. Die Entwicklung wurde in höheren Konzentrationen nur schwach verzögert befunden. Es bestanden keine signifikanten Unterschiede zwischen den Sterblichkeitsraten bei verschiedenen Konzentrationen, nur in der höchsten Konzentration (5% Salzlösung) zeigte sich eine höhere Mortalität.

Die Empfindlichkeit von *Aedes togoi* und zwei Stämmen von *Aedes aegypti* wurde mit den W.H.O.-Prüfwannen gemessen. Die Imagines wurden mit DDT und die Larven mit DDT, Dieldrin und gamma-BHC geprüft. Der vorliegende Stamm von *Aedes togoi* entstammte einem Gebiet, das von überständigem Antimalaria-DDT-Gebäudesprührückstand bedeckt war; so konnte die Möglichkeit einer Resistenzentwicklung bei diesen Mücken erwartet werden. Unglücklicherweise ist kein Stamm von *Aedes togoi* verfügbar von Orten, die von DDT-Begiftung frei sind; deshalb kann seine mögliche Resistenz gegen DDT nicht abgeschätzt werden. Jedoch erwies er sich als ebenso empfindlich wie normale Stämme von *Aedes aegypti* aus dem südlichen Taiwan und Pescadores. Die männlichen Imagines von *Aedes togoi* sind viel stärker DDT-empfindlich als die Weibchen und beide Geschlechter normaler Stämme von *Aedes aegypti.* Im Larvalstadium ist *Aedes togoi* viel toleranter gegen DDT als normale Stämme von *Aedes aegypti.* Die Resulte sind in 12 Tabellen und in den Figuren 1 und 2 aufgeführt.

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OBSERVATIONS ON THE PALATABILITY AND UTILISATION OF FOOD BY LOCUSTS, WITH PARTICULAR REFERENCE TO THE INTERPRETATION OF PERFORMANCES IN GROWTH TRIALS USING SYNTHETIC DIETS

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The phagostimulant effectiveness of substances used in artificial diets for *Schistocerca* was assessed by comparing the amounts of faecal material produced by hungry hoppers allowed to feed on filter paper soaked in solutions of the substances with the amounts produced when water alone was used on the paper. Sucrose, glucose and wheatgerm oil caused markedly greater faecal production than distilled water alone. Chlorophyll, carotene, casein, peptone, olive oil, a nutritional salt mixture, a mixture of B vitamins, choline chloride and ascorbic acid were no more effective than water. Aqueous extracts of yeast were generally as effective as sugar but sometimes seemed inhibitory at high concentrations. As growth could occur on diets lacking yeast, sugar and wheatgerm oil, the only dietary components found to have pronounced phagostimulatory properties, it appears that special gustatory stimuli are unnecessary for initiating feeding activity in hungry hoppers. This view was later borne out in experiments in which the amounts of various complete diets eaten and the faeces produced from them were compared. No major differences in feeding attributable to palatability were apparent, but the amounts of food taken were found to be related to differences in utilisability. With both *Schistocerca* and *Locusta,* the lower the utilisability of the food the greater the amount eaten and for a range of diets of various utilisabilities, including fresh grass, the values obtained by multiplying the amount eaten (dry weight) by the coefficient of utilisation were similar for each species. It is suggested that in locust hoppers special gustatory stimuli are unnecessary for the initiation of feeding and of minor importance in sustaining it. The amount of food taken appears to be largely regulated by its overall utilisability where food of more or less adequate nutritional composition is concerned, a situation not wholly unexpected in insects able to thrive on diverse food plants.

Implicit in most studies on insect nutrition is the assumption that where poor growth results from the omission of particular components from a satisfactory synthetic diet the components in question are essential nutrients. This is doubtless so in most cases, but quite clearly poor growth could occur if a deficient component were a phagostimulant whose omission led to a reduction in feeding, a possibility rarely considered.

The importance of phagostimulation in normal feeding has been most clearly shown in work with various oligophagous lepidopterous larvae. In addition to specific attractants, which are often essential oils (DETHIER, 1947) enabling the larvae to locate and bite their food, specific phagostimulant substances must be present in the food, as they always are in the natural diet, if sustained feeding

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is to occur (Dethier, 1937, 1941; Thorsteinson, 1953a; De Wilde, 1958; Hamamura, 1959). The dearth of nutritional studies on phytophagous insects, many of which are oligophagous, stems from failure to induce feeding on artificial diets, the failure being due to ignorance of the identity of attractant and phagostimulant substances in most cases.

In polyphagous insects a variety of foods are able to elicit sustained feeding, and the stimuli concerned are clearly of low specificity and probably of complex origin. If the latter is the case it may be envisaged that degrees of sub-optimal stimulation might be associated with synthetic diets embodying only some of the total sources of partial stimulation, which integrated, would provide optimum palatability. In this type of situation poor performances in growth trials brought about by omission of substances from artificial diets could only be confidently accepted as evidence of a nutritional requirement if it was clear that the diet had been eaten in adequate amounts. It can reasonably be inferred that normal feeding has occurred in growth trials if failure is preceded by an initial period of good growth. Confusion arises where moderate but sub-optimal growth occurs from the start.

In developing synthetic diets for use with locusts this type of suboptimal growth was for long a barrier to progress, and the problems involved have been discussed at length elsewhere (Dadd, 1960a). When a satisfactory diet was finally devised it appeared that the early failures were neither primarily nutritional nor due to poor palatability, but resulted from an imbalance in the composition of diets, particularly a lack of indigestible bulk material. In arriving at this conclusion the phagostimulant properties of a number of substances used in artificial diets were tested, and detailed studies on the ingestion of complete diets were made. These observations were ancillary to growth trials of diets, and when a satisfactory formulation was developed they were discontinued. The results obtained are described in this account, for although incomplete they illuminate an important but neglected aspect of nutritional studies.

METHODS

Most of this work relates to the desert locust *Schistocerca gregaria* (Forsk.), as only this species could be reared on the yeast-containing diets at first available. With improved synthetic diets the migratory locust, *Locusta migratoria* L. became amenable to study, and some of the experiments on the ingestion and utilisation of complete diets dealt with this species.

Two types of experiments are described. The phagostimulant effect of single dietary components was tested by a method similar to those of Thorsteinson (1953b) and Chauvin (1951). Discs of filter paper were soaked in solutions (or suspensions in some cases) of the test substances and and excess fluid drained off. The papers were pinned to large corks and placed at the bottom of 1½ litre glass jars into which hungry hoppers were placed. These hoppers had been kept overnight without food although with drinking water; after this period their guts were emptied of contents and they rarely thereafter passed faecal pellets containing grass. Where lipids were tested the papers were first soaked in appropriate ethereal solutions, the ether evaporated off and the papers re-soaked in distilled water. After 24 hours in a room held at 32 ± ½° C and 55 ± 5% R.H. any faecal pellets produced were counted and weighed. The latter value was taken to be an index of the amount of paper eaten, for although notes were made on the extent of visible eating of the filter papers, it was not practicable to measure this directly in terms of the area eaten as Thorsteinson had done, for considerable surface scraping occurred. Substances were considered to stimulate feeding if greater quantities of faeces were obtained after treatment with their solutions than with distilled water alone.

Groups of second instar hoppers were used in a few initial experiments, but subsequently fourth or fifth instar hoppers were tested in isolation, thereby affording some indication of the variability of the feeding responce. Hoppers were kindly supplied from gregarious stocks of the Anti-Locust Research Centre. Care was taken to select them in mid-instar, for with late-instar hoppers negative results might have been due to the onset of moulting, when feeding normally diminishes (Davey, 1954). If in spite of this moulting occurred during the course of an experiment, or if hoppers died, the particular result was discarded.

In experiments on the ingestion of complete diets early fifth instar hoppers kept without food overnight were used in tests lasting three days. As before, this preliminary starvation ensured both a state of hunger and that the alimentary tract was emptied of grass.

After starvation, hoppers were individually weighed and placed in groups of five in 12 litre celluloid cages. Each cage had a perforated zinc perch, a dish containing soaked cotton wool for drinking water and a petri dish containing an accurately weighed amount of diet. On setting out diets weighed samples were taken. These were dried at 60° C for 24 hours, and after re-weighing, provided a conversion factor for calculating the dry weight of diet dispensed. It was found that further loss in weight on drying at 100° C was negligibly small, both with synthetic diets and grass, and all dry weights were taken after 24 hours in a 60° C incubator. Whenever possible sufficient diet for the three-day experiment was dispensed at the start. Occasionally replenishment of synthetic diets was required, and grass was supplied afresh each day; on these occasions additional samples were taken for the calculation of dry weights.

After setting up an experiment all cages were left in a Constant Temperature room at 32 ± ½° C and 55 ± 5% R.H. for 24 hours. Hoppers were then re-weighed, all faecal pellets were collected, counted and dried prior to weighing, and the cages returned to the C.T. room for a further 24 hours. Where grass was used, all remnants were collected every day before providing fresh supplies, dried and weighed.

This procedure was followed for three days. On completion of the third day all diet remaining was carefully collected from each cage, dried and weighed. Hoppers were then returned to their cages, now empty except for their water supply, and left for a further 24 hours, after which pellets derived from food remaining in the gut were collected, dried and weighed. In the first two experiments with *Schistocerca* this final collection of faecal pellets had not been introduced, and consequently faecal weights were slightly low and coefficients of utilisation somewhat high. However, the data of later experiments indicated that this omission resulted in differences of only 5% in faecal weights and 2—4% in coefficients of utilisation.

From the data thus recorded mean daily increments in hopper weight and mean daily dry weights of faecal pellets were calculated for each group of five hoppers. By subtracting the dry weight of residual food from the calculated dry weight of dispensed food and dividing by the number of hoppers per group, the mean weight of food eaten per hopper over three days was determined. A coefficient of utilisation, defined as "dry weight of food eaten minus dry weight of faeces produced, divided by dry weight of food eaten", (EVANS, 1939; TRAGER, 1953) was calculated for each group.

Occasionally hoppers died during the course of an experiment. If more than one died, or if extensive cannibalism of the corpse had occurred, the results for the group were discarded. Otherwise means for the 24 hours during which death had occurred were worked out on the basis of four hoppers, and in calculating the amount of food eaten per hopper over three days a suitable adjustment was made. For example, if death occurred on the second of the three days, the observed dry weight of food eaten was divided by 4½ instead of the usual 5, the dead hopper being considered to have eaten half the amount it would have done had it survived the full period. Where such adjustments were made a note to this effect is included in the tables of results.

RESULTS

In initial experiments various substances were tested with a view to finding something which, compared with water, induced sufficiently marked feeding to be suitable for use in subsequent tests as a positive control. Sugars were tried, as they are known to induce feeding in many insects, and olive oil because it was reported to stimulate feeding with adult *Schistocerca* (CHAUVIN, 1951). "Cerophyll" is a proprietary preparation of dried grass juice, and might be supposed to contain specific phagostimulant factors occurring in normal food material, should such be of importance. Chlorophyll and carotene, both characteristically present in green plant tissues, were tested because at the time they were suspected of improving the growth of *Schistocerca* on artificial diets and it was thought that this might be due to improved palatability. Groups of five second instar hoppers were used in these initial tests, and the extent of feeding, as shown by the amounts of faeces produced, is indicated in Table I.

TABLE I

Dry Weights (mg) of faeces produced in 24 hours by groups of five second-instar hoppers of Schistocerca *offered filter papers soaked in various solutions.*

	Exp. A	Exp B	Exp C
Tap water	7		
Distilled water	11	8	10
Carotene (25 mgs in 100 mls ether) + water	13		
2% sucrose	52	35	43
2% sucrose + carotene	50		
2% soluble starch		34	
2% maltose			22
2% lactose			60
1% water-soluble chlorophyll paste		6	12
1% chlorophyll + 2% sucrose		37	43
10% cerophyll		3	0
Olive oil, (10% in ether)		7	8

Two points emerged. Some faecal pellets were produced with all groups where water alone was used, and examination of the appropriate filter papers showed extensive nibbling. It appeared therefore that hunger alone would induce *Schistocerca* hoppers to feed. However, feeding was markedly more extensive with carbohydrate solutions, and 2% sucrose was considered a suitable positive control for subsequent use.

Chlorophyll, carotene, and olive oil were without apparent effect, and rather unexpectedly the grass juice preparation "Cerophyll" was rejected; no faeces containing paper were produced, and no nibbling could be detected on the filter papers.

TABLE II

Dry weights of faeces produced by Schistocerca *hoppers offered filter papers soaked in sugar solutions.*

		Individual faecal weights (mg)		Mean
Experiment D (4th instar) *	distilled water	5, 6, 10		7
	water + rutin (saturated) (solution)	6, 14, 6		9
	2% sucrose	67, 46, 26		46
	2% sucrose + rutin	49, 57, 46		51
Experiment E (5th instar) *	distilled water	82, 35, 17, 32, 58		45
	water + rutin	49, 62, 14, 48, 30		41
	2% sucrose	124, 96, 73, 122, 86		100
	2% sucrose + rutin	98, 177, 60, 121, 180		127
	2% glucose	136, 158, 96, 77, 102		114
	2% glucose + rutin	129, 166, 90, 64, 83		106
Experiment F (5th instar)	distilled water	34, 133, 4	(1 moulted)	57
	2% sucrose	216, 248, 262	(1 moulted)	235
	2% fructose	247, 302, 212	(1 moulted)	277
	10% glycerin	46, 44, 47	(1 moulted)	46
Experiment G (4th instar)	distilled water	5, 3, 2, 2, 6		4
	0.125% sucrose	4, 13, 9, 13, 19		12
	0.25% "	6, 20, 1, 25, 38		18
	0.5% "	1, 26, 22, 22, 38		22
	1.0% "	58, 31, 37, 32	(1 moulted)	40
	2% "	72, 86, 103, 18, 48		65
	4% "	74, 18, 84, 146	(1 moulted)	81
	8% "	23, 6, 2, 20, 31		16
Experiment H (5th instar)	distilled water	44, 23, 29, 37, 19		30
	0.03% fructose	37, 33, 40, 31, 7		30
	0.25% fructose	74, 36, 70, 63, 35		56
	2.0% fructose	146, 134, 195, 118, 153		149
Experiment I (5th instar)	water	60, 45, 43, 84		58
	0.015% soluble starch	83, 42, 43, 64		58
	0.063% " "	35, 19, 45, 20		30
	0.25% " "	48, 40, 58, 58		51
	1.0% " "	96, 101, 166, 127		123
	2.0% " "	120, 59, 112, 151		111

* The flavone glycoside rutin was tested in these experiments as its inclusion in artificial diets had seemed to improve growth in some early trials. It has subsequently been shown to have no effect on growth, and in these experiments *Schistocerca* appears indifferent to its presence.

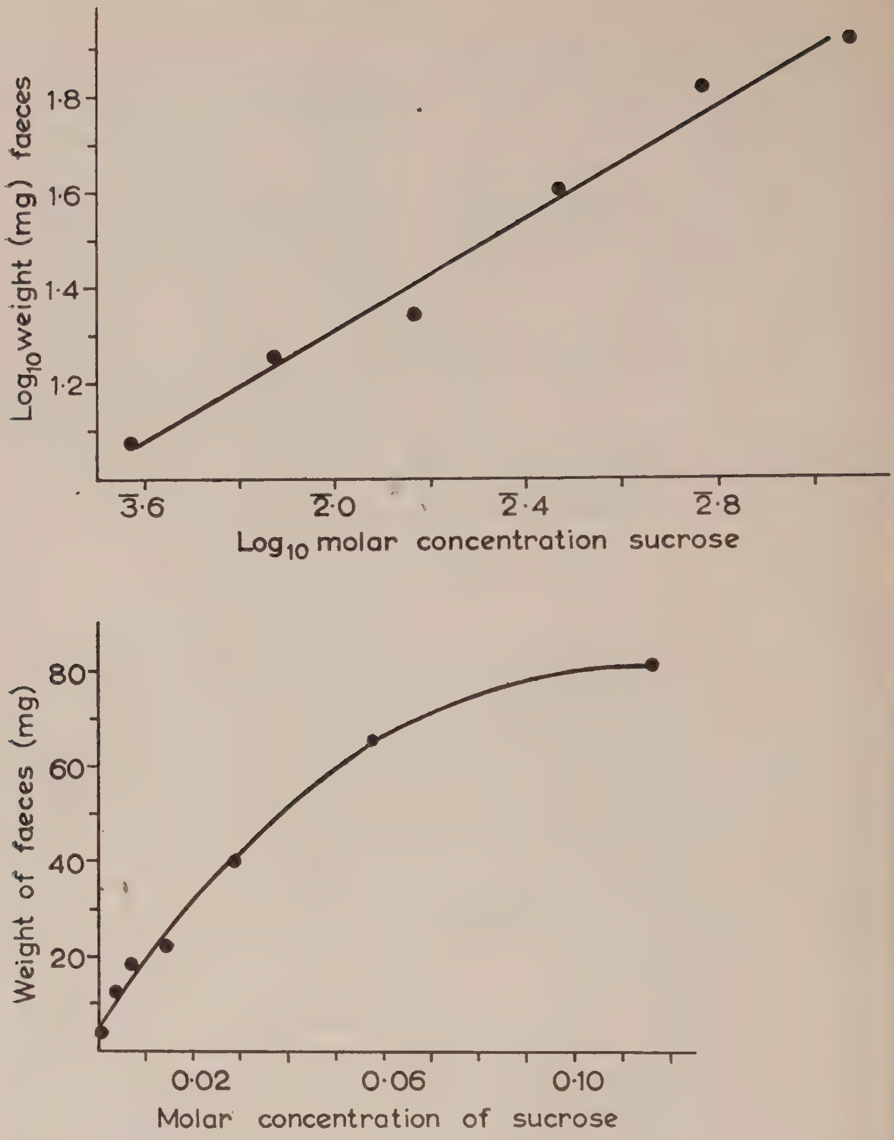

Fig. 1. Graph based on the data of Table II, experiment G, to illustrate the relationship between the amount of filter paper eaten by fourth instar *Schistocerca* hoppers (in terms of weight of cellulose egested as faecal pellets) and the concentration of sucrose solutions with which the filter paper was moistened.

The effect of sugars on feeding was further examined in a series of experiments whose results are given in Table II. Isolated fourth or fifth instar hoppers were used so that individual variability could be appreciated. Quite clearly solutions of sucrose, glucose, fructose and soluble starch caused much more feeding, on average, than did water alone, although individual results varied widely.

The occasional very low result with sugar (e.g. in the 2 and 4% sucrose groups of experiment G) might be accounted for in terms of the normal low level of feeding activity at the beginning and end of each instar (DAVEY, 1954), for although an attempt was made to exclude hoppers in these conditions some may have been used inadvertently; as moulting occurred from time to time during the course of an experiment, late instar hoppers must have been present in the samples taken on such occasions.

As with second instar hoppers, some faecal production generally occurred where water only was used. Individual hoppers fairly frequently produced considerable amounts of faeces (and the corresponding filter papers were extensively eaten) and it is clear that by itself hunger may cause extended feeding. This being so, it could be expected that only strongly phagostimulant substances such as the sugars would give clearly positive results in tests using small numbers of hoppers.

Some idea of the sensitivity of the method in distinguishing degrees of phagostimulation is given by the results for a test using different concentrations of sucrose (Table II, G). Doubling the concentration in steps from 0.125% to 4% gave a regular increase in the average weight of faeces produced, while at 8% a sharp decrease was obtained. The results for values up to 4% sucrose are represented graphically in Fig. 1. It will be seen that when mean weights of faeces are plotted directly against molar concentrations of sucrose the curve obtained approaches a flat maximum at about 0.1 M. If the logarithms of mean faecal weigths are plotted against the logarithms of molar concentration, the curve obtained for values up to the maximum is linear. Linear relationships of this sort are well known from studies of the ingestion of sugar solutions by adult insects (DETHIER, 1953b) and have been demonstrated, using complete diets, in the larvae of *Pyrausta* (BECK, 1956b).

Taking this experiment as a whole it can be seen that quite small differences in the concentration of a phagostimulant substance may be distinguished, although taken alone the small differences in faecal production between the lower concentrations would not be significant with the numbers used. The low faecal weight obtained on the highest concentration of sucrose indicated that if present in excess, phagostimulatory substances might lose their effectiveness.

Quite clearly, tests on the scale here employed could not detect weakly phagostimulant effects. However, as the intention was to assess the importance of substances used in artificial diets in contributing to overall palatability, interest mainly centred on those which might have marked phagostimulatory properties, for as sucrose was used in all diets, other components were unlikely to greatly affect palatability unless they had comparable powers of phagostimulation or inhibition. Extensive tests were therefore limited to substances for which it was especially important to have information on their "palatability status" because of doubtful effects in growth trials.

Data for tests with some of the components of artificial diets are given in Table III. As the casein (B.D.H. fat and vitamin free) was insoluble and the salt mixture only partially soluble, they were applied to the filter papers as suspensions after thorough shaking. The solution of B vitamins had the composition listed in Table VII with the omission of choline chloride.

Only wheatgerm oil gave results comparable with sucrose. On the limited data available, little significance attaches to the slightly greater amounts of faeces

TABLE III

Dry weights of faeces produced by Schistocerca *hoppers offered filter papers treated with some substances used in artificial diets.*

		Individual faecal wts (mg)	Means
Experiment J (5th instar)	tap water	54, 88, 33, 15, 47	47
	2% sucrose	179, 136, 174, 112, 161	152
	2% peptone	96, 111, 6, 83, 3	60
	2% casein (suspension)	88, 53, 5, 34, 79	52
	10% wheatgerm oil	82, 92, 114, 143, 111	108
	10% olive oil	47, 120, 81, 35, 45	66
	10% glycerine	24, 56, 50, 65, 69	53
Experiment K (5th instar)	distilled water	17, 20, 52, 51, 22	32
	B vitamin mixture	53, 74, 41, 6	(1 dead) 44
	2% sucrose	164, 222, 190, 208, 298	216
	2% sucrose + vitamin mixture	231, 166, 228, 176, 227	206
	1% salt mixture (Steenbocks No. 140)	21, 0, 25, 7, 43	19
	2% sucrose + 1% salts	173, 212, 184, 229, 248	209

produced with peptone, olive oil and the vitamin mixture, or to the apparently inhibitory effect of the salt mixture. When tested in combination with 2% sucrose the latter two components were without effect.

Two water soluble vitamins, ascorbic acid and choline chloride, were dealt with individually in greater detail because of their importance in growth trials with artificial diets. Ascorbic acid, an essential dietary component for *Schistocerca* (DADD, 1957), was found to have phagostimulant properties for the grasshopper *Chorthippus* (THORSTEINSON, 1956). It has subsequently been shown that for *Schistocerca* the dietary requirement is nutritional in the strict sense, and relevant phagostimulation tests are described with this work (DADD, 1960c). Over a wide range of concentrations no effect on faecal production could be found, either alone or in combination with sucrose.

Choline chloride was found to improve growth when included in yeast-containing diets, but as a clearcut requirement for it could not at first be demonstrated, an effect on palatability was suspected. Data from tests with choline chloride are given in Table IV. It clearly has little effect, except perhaps for some slight inhibition of feeding at very high concentrations. Subsequent work has shown this substance to be an essential nutrient.

A series of experiments were devoted to the effect of yeast on feeding. Yeast was included in early artificial diets primarily as a source of vitamins, but the first attempts to replace it by vitamins and additional protein (casein) were unsuccessful, a situation which might have resulted were yeast an important stimulant in feeding. As ether-extracted yeast could be used in diets without deleterious effect on growth, it was assumed that any important phagostimulatory property was likely to derive from the water-soluble fraction. Aqueous extracts of yeast were therefore tested. In all experiments these were prepared immediately before use by boiling 6 g of yeast in 100 ml of distilled water for 15 minutes, centrifuging down the solids and using the clear supernatant.

TABLE IV

Dry weights of faeces produced by Schistocerca *hoppers offered filter paper soaked in solutions of choline chloride*

		Individual faecal wts (mg)	Means
Experiment L (5th instar)	distilled water	13, 3, 6, 12, 15	10
	0.125% choline chloride	4, 24, 14, 6, 8	11
	2% choline chloride	10, 20, 30, 10, 3	15
	2% sucrose	100, 77, 86, 66, 105	87
	2% sucrose + 0.125% choline chloride	51, 75, 53, 25, 121	65
	2% sucrose + 2% choline chloride	54, 54, 64, 39, 50	52
Experiment M (5th instar)	2% sucrose	167, 154, 94, 145, 170 211, 97, 190, 187, 189	160
	2% sucrose + 0.03% choline chloride	167, 114, 114, 187, 124 174, 161, 152, 180, 194	157
	2% sucrose + 0.5% choline chloride	113, 134, 135, 133, 180 195, 74, 177, 136 (1 moulting)	142

Data from experiments using such extracts are given in Table V. It is apparent that, in general, more faeces were produced with yeast extracts than with water alone, but quite clearly the concentration of the extract was crucial. Thus, in experiment N the mean value for the concentrated extract was no greater than for water; halving and quartering the concentration gave progressively higher weights of faeces, in the latter case approaching those obtaining with 2% sucrose, while further dilution diminished the effect. In a corresponding series using a debittered-yeast extract (Exp. Q) results were similar except that faecal weights increased with dilution over the whole range tested.

A curious situation is revealed by data for combinations of bitter yeast extract and sucrose (Experiments O and P), for in all cases less faeces were produced than for corresponding sucrose controls. As yeast extracts alone were more effective than water, combination with sucrose might have been expected either to increase faecal production, or to be without apparent effect if phagostimulation from sugar alone was optimal. A result of this sort was obtained with debittered yeast in experiment R.

A further point of interest arises from a consideration of the individual data for bitter yeast extracts. In many instances filter papers remained completely uneaten, whereas those individuals which ate, did so as extensively as with sucrose. Generally speaking, where faecal weights of less than 10 mg were recorded with yeast extracts, papers were untouched; with water alone, papers characteristically showed numerous small bite marks, even when scarcely eaten.

These results may be interpreted by supposing that yeast has both acceptant and rejectant properties, the balance beween these, both alone and in relation to other phagostimulants such as sucrose, being affected by concentration. From the extreme variation in individual results with bitter yeast it would appear that rejection functioned in an all or none way, and that where individual hoppers

TABLE V

Dry weights of faeces produced by Schistocerca *nymphs offered filter papers soaked in aqueous extracts of yeast*

		Individual faecal wts (mg)	Means
Experiment N	distilled water	44, 29, 28, 26, 19	29
	2% sucrose	153, 144, 89, 213, 80	136
	Bitter yeast extract full strength	0, 5, 15, 109, (1 dead)	32
	Bitter yeast extract half strength	163, 211, 4, 3, 0	76
	Bitter yeast extract quarter strength	152, 129, 3, 80, 128	104
	Bitter yeast extract eighth strength	91, 13, 113, 12, 19	50
Experiment O	water	29, 24, 33, 4	23
	Bitter yeast extract full strength	8, 178, 4, 78	67
	Bitter yeast extract quarter strength	2, 198, 72, 0	68
	2% sucrose	54, 64, 138, 118	94
	2% sucrose + full strength yeast extract	46, 33, 128, 0	52
	2% sucrose + quarter strength yeast extract	65, 4, 67, 80	54
Experiment P	water	48, 47, 39, 11, 86, 52, 58	49
	Bitter yeast extract	79, 53, 23, 241, 91, 78, 54	88
	2% sucrose	226, 208, 165, 168, 195, 140, 130	176
	2% sucrose + yeast extract	100, 185, 78, 156, 192, 264, 191	142
Experiment Q	distilled water	60, 59, 64, 30, 30	49
	2% sucrose	175, 78, 176, 187, 117	147
	debittered yeast extract full strength	34, 68, 18, 164, 12	59
	debittered yeast extract half strength	47, 23, 126, 37, 233	93
	debittered yeast extract quarter strength	163, 186, 27, 57, 90	105
	debittered yeast extract eighth strength	105, 159, 142, 70, 174	130
Experiment R	distilled water	0, 10, 25, 13, 21	17
	debittered yeast extract	132, 30, 95, 25, 145	85
	debittered yeast extract eighth strength	74, 74, 0, 20, 6	43
	2% sucrose	133, 145, 110, 151, 0	108
	2% sucrose + full extract	63, 138, 4, 93, 184	96
	2% sucrose + eighth strength extract	50, 208, 186, 126, 209	156

fed, they did so as extensively as with sugar. In so far as debittered yeast seemed to possess weaker rejectant powers, it is reasonable to suppose that these may be connected with the bitter principle of hops, largely washed out in the course of debittering brewer's yeast.

Two final experiments dealt with "Cerophyll" and an ether extract of bran.

"Cerophyll" was retested because improved growth of *Schistocerca* resulted from its incorporation into yeast-containing artificial diets and it was difficult to reconcile this with the apparent inhibition of feeding noted earlier. A very high concentration of Cerophyll was used in the early experiments; in this case low concentrations were tested. In testing the extract of bran it was hoped to determine whether this preparation was as powerful a phagostimulant for the nymphs of *Schistocerca* as it had been for the adults (CHAUVIN, 1951). The extract was prepared in the cold as described by Chauvin and used at two concentrations. Results for these experiments are given in Table VI.

TABLE VI

Dry weights of faeces produced by Schistocerca *hoppers offered filter papers treated with Cerophyll solutions or an ether extract of bran*

		Individual faecal wts (mg)		Means
Experiment S (5th instar)	distilled water	0, 21, 44, 65, 11, 12, 9, 21, 7	(1 moulted)	21
	2% sucrose	118, 50, 62, 35, 40, 159, 47, 60	(2 moulted)	71
	2% Cerophyll	25, 58, 21, 100, 74, 25, 56, 69	(2 moulted)	54
	1% Cerophyll	5, 26, 9, 29, 26, 34, 25, 25	(1 moulted)	23
	0.5% Cerophyll	14, 7, 18, 44, 22, 32, 20, 44	(1 moulted)	26
	0.25% Cerophyll	16, 59, 14, 12, 9, 11, 31, 39	(2 moulted)	24
Experiment T (5th instar)	distilled water	5, 10, 17, 56, 9		19
	2% sucrose	79, 114, 55, 110, 132		98
	ether extract of bran full strength	135, 177, 78, 67, 48		101
	ether extract of bran diluted to one tenth strength	132, 38, 53, 73, 22		64

At concentrations of 0.25, 0.5 and 1.0%, "Cerophyll" was no more effective than water, but at 2% a marked increase in faecal production was obtained, although less than with 2% sucrose. This suggests that the inhibition of feeding which occurred in the preliminary experiments was a result of the very high concentration (10%) then used. The ether extract of bran proved to be markedly phagostimulant at both concentrations, the higher being as effective as sucrose. Chauvin ascribed the well-known attractiveness of bran for locusts to this ether soluble fraction, and it appears that the same mechanism operates with the immature stages. In this connection it may be noted that of the various components of synthetic diets to be tested, the only one to show a phagostimulant effect comparable with sugar was wheatgerm oil, which has obvious affinities with the ether-soluble fraction of bran.

These phagostimulation tests proved of little help in developing a satisfactory diet, and progress in this direction remained dependent on trial and error methods. When the question of palatability again assumed importance, substances suspected of stimulating feeding activity were tested for an effect on ingestion in complete diets. The occasion for such studies arose when it was found that yeast-free synthetic diets were much improved by the incorporation of crude grass protein, a preparation kindly supplied by the Grassland Research Station, Hurley, Buckinghamshire. This improvement might have been nutritional or a result of improved palatability. The latter possibility was examined by comparing the amounts of grass and synthetic diets, with and without grass protein, eaten by fifth instar hoppers during a three day test period.

These comparisons, results of which are summarised in Table VIII, experiments A and B, showed that the synthetic diets then being used were grossly deficient in indigestible material when compared with grass. Incorporation of grass protein reduced utilisation, presumably because this preparation was only partly digestible, and it was supposed that growth on such diets might have been improved for this reason. The crucial importance of this information in developing a satisfactory basal diet for use in evaluating nutritional requirements has been fully discussed elsewhere (DADD, 1960a).

TABLE VII

The composition of some synthetic diets used in experiments on food ingestion and utilisation

		A	B	C	D	E
Cellulose powder	(g)	5	5	15	10	20
wheatgerm oil	(ml)	1	1	1	1	1
β-carotene	(mg)	25	25	25	25	25
salt mixture	(g)	1	1	1.5	1.5	1.5
sucrose	(g)	15	15	5	5	5
dextrin	(g)			5	10	3.0
Casein	(g)	6	4	6	6	4.5
peptone	(g)	2		2	2	1.5
egg albumen	(g)	2		2	2	1.5
crude grass protein	(g)		12			
ascorbic acid	(mg)	100	100	100	100	100
B vitamins in 10 ml water:						
thiamin	μg/g diet	25	25	25	25	25
riboflavin	μg/g diet	25	25	25	25	25
nicotinic acid	μg/g diet	50	50	100	100	100
pyridoxine	μg/g diet	25	25	25	25	25
folic acid	μg/g diet	25	25	25	25	25
inositol	μg/g diet	250	250	250	250	250
calcium pantothenate	μg/g diet	50	50	50	50	50
p-aminobenzoic acid	μg/g diet	25	25	25	25	25
biotin	μg/g diet	1	1	1	1	1
choline chloride	μg/g diet	1250	1250	1250	1250	1250

Subsequently various modifications of this basal diet were used in further ingestion experiments. These were mainly concerned to determine whether grass was eaten in greater quantities than synthetic diets, and whether substantially differing amounts of different diets were taken. Data for experiments with *Schistocerca* are given in Table VIII, and for *Locusta* in Table IX. The composition of diets referred to in these experiments is given in Table VII.

A striking point to emerge is the general similarity in the mean weight increments of hoppers after both 24 hours and 3 day periods of feeding, regardless of the food. In so far as the increase in weight after 24 hours can be taken as an index of the amount of food eaten, the data provide no indication that grass was

TABLE VIII

Data from experiments with Schistocerca *hoppers on the amount of food eaten, the weight of faeces produced, and the utilisation of the food*

	Experiment	Bran	Grass	Synthetic diets with very low cellulose (16%) (table VII, A)	Grass protein diet (table VII, B)	Standard Syn. diet (cellulose 40%) (table VII, C)	Low cellulose diet (cellulose 26%) (table VII, D)	High cellulose diet (cellulose 53%) (table VII, E)	Standard synthetic diet + 1% chlorophyll
Mean Weight increase of hoppers after 24 hrs. (mg)	A	376	229, 302	257, 115	324, 469				
	B		270, 235	314, 261	356, 271	241, 281			
	C		222, 284			277, 333	351, 288		328, 335
	D		101, 106		258, 261	211, 249	204, 334		293, 327
	E		309			343, 266	294, 328	277, 209	
	Average	376	229	237	323	275	300	243	321
Mean Weight increase of hoppers after 3 days (mg)	A	530	447, 680	623, 233	433, 650				
	B		446, 659	430, 506	626, 606	554, 482			
	C		646, 662			683, 645	725, 691		699, 705
	D		401, 396		685, 639	588, 620	636, 728		729, 769
	E		613			704, 638	694, 729	636, 586	
	Average	530	550	448	607	614	701	611	726
Dry Weight of faeces produced per hopper in 3 days (mg)	A	513	436, 610	96, 51	125, 186				
	B		359, 530	70, 80	199, 213	248, 219			
	C		526, 640			437, 395	286, 273		465, 425
	D		501, 506		285, 337	390, 419	274, 318		425, 475
	E		443,			403, 404	259, 283	571, 600	
	Average	513	506	74	224	364	282	586	448
Dry weight of food eaten per hopper in 3 days (mg)	A	851	684, 1068	324, 210	422, 566				
	B		499, 921	327, 331	542, 638	509, 456			
	C		875, 1052			791, 736	667, 651		856, 808
	D		802, 790		708, 751	708, 749	502, 702		774,* 887
	E		943,			788, 775	639, 701	920, 917*	
	Average	851	848	298	605	689	644	919	831
Coefficients of utilisation	A	40%	36%, 43%	70%, 76%	70%, 67%				
	B		28%, 42%	79%, 76%	63%. 67%	52%, 52%			
	C		40%, 39%			45%, 44%	45% 55%		46%, 47%
	D		38%, 36%		60%, 55%	45%, 46%	57%, 58%		45%,* 46%
	E		53%			49%, 48%	59% 60%	38%, 35%	
	Average	40%	39%	75%	64%	48%	56%	37%	46%

*One hopper died during the experiment.

TABLE IX

Data from experiments with Locusta *hoppers on the amount of food eaten, the weight of faeces produced and the utilisation of the food*

	Exp.	Grass	Standard Syn. diet (cellulose 40%) (Table VII C)	Low cellulose diet (cellulose 26%) (Table VII, D)	High cellulose diet (cellulose 53%) (Table VII E)	Standard diet without wheat-germ oil	Standard diet with low oil	Standard diet with double amount of oil	Standard diet without Carotene	Standard diet with low Carotene
Mean Weight increase of hoppers after 24 hrs (mg)	A	193, 212	221, 235	258, 305	175, 261					
	B	204, 262	292, 326			322, 309	297, 325	302, 336		
	C	178, 160	247, 279						240, 292	267, 311
	Average	212	267	282	218	316	311	319	266	289
Mean Weight increase of hoppers after 3 days	A	587, 591	429, 506	514, 561	461, 509					
	B	518, 622	589, 577			480, 554	566, 538	578, 581		
	C	414, 432	453, 437						436, 448	517, 488
	Average	527	499	538	485	517	522	580	442	503
Dry Weight of faeces produced per hopper in 3 days	A	390, 388	257, 289	209, 251	539, 537					
	B	533, 592	374, 349			257, 376	415. 395	382, 398		
	C	452, 518	310, 317						307, 343	393, 296
	Average	479	316	230	538	317	405	391	325	345
Dry Weight of food eaten per hopper in 3 days	A	751, 763	463, 497	467, 534	856, 819					
	B	884, 868	700, 652			493, 627	709, 817	706, 717		
	C	633,* 811*	584, 548						627, 572	728,* 562
	Average	785	574	501	838	560	714	712	600	645
Coefficients of utilisation	A	48%, 49%	45%, 42%	55%, 53%	37%, 35%					
	B	40%, 32%	47%, 46%			49%, 40%	41%, 45%	46%, 44%		
	C	29%,*36%*	47%, 42%						51%, 40%	45%,*47%
	Average	39%	45%	54%	36%	45%	43%	45%	46%	46%

* One hopper died during experiment.

preferred. However, in a few experiments weight increments after 2 hours with food were recorded (not given in the tables), and generally speaking these were lower for synthetic diets than for grass, although the distinction was not clear-cut. These data would more closely reflect the immediate acceptability of food, for complications due to loss of faeces and conversion to tissue material would be negligible; to this extent they indicated that there was probably a greater readiness to eat grass. But quite clearly this immediate effect is overshadowed within 24 hours, and at 3 days one has the curious situation with *Schistocerca* that, generally speaking, more growth occurred on synthetic diets than on grass.

In contrast to the similarity in weight increments shown by hoppers on all foods, the values for the dry weight of food eaten and the dry weight of faeces produced vary widely. Thus with *Schistocerca* the average amount (dry weight) of grass eaten was three times that of the early synthetic diet A, and about 50% greater than the grass-protein modification; faecal production on grass was seven times that on the synthetic diet and over twice that on the grass protein diet. In view of the similarities in hopper growth during the period of the test, implying that similar quantities of *nutrient* material were utilised, this disparity suggested that the utilisability of the foods in question must be very different. Calculation of coefficients of utilisation showed this to be so.

Results for synthetic diets containing various proportions of cellulose show that, as might be expected, those with more cellulose produce greater amounts of faeces. Of greater interest is the finding that the amounts of diet eaten increased with increased content of cellulose. The diet containing 53% of cellulose, thereby having a coefficient of utilisation (37%) similar to the average found for grass (39%), was eaten in quantities of the order found for grass. Although the data are limited, it will be seen that a similar relationship between cellulose content, faecal production and amount of food ingested also holds for *Locusta.*

These relationships implied that, with diets of more or less nutritional adequacy, utilisability plays an important role in regulating feeding. With this idea in mind it seemed worthwhile comparing values representing the amount of *utilisable* food eaten for the various foods used. Such values were obtained by multiplying the dry weights of diet eaten by corresponding coefficients of utilisation, and are given in Table X.

The average values thus obtained for *Schistocerca* are remarkably similar, falling in the range 325—383, with the exception of the low value of 224 for the early synthetic diet A. It will be recalled that it was the unsatisfactory performances obtained in growth trials using this diet that led to the present investigation. As all the other diets used in these experiments gave more or less similar performances in growth trials and were only slightly inferior to grass in supporting growth to the adult stage, it seems that with satisfactory foods the dry weight of food taken is inversely proportional to its utilisability.

Several modifications of the basic synthetic diet, all with 40% cellulose, were subsequently studied. Data for experiments using some of them with *Locusta* are given in Table IX. These results are essentially similar to those obtained with the basic synthetic diet, and the same was true of identical experiments using *Schistocerca.* Values calculated for the amount of utilisable food ingested by *Locusta* (Table X) are fairly similar considering the limited data on which they

TABLE X

Calculated values for the weights of utilisable food ingested per hopper during three days (mg)

Exp.	Bran	Grass	Early synthetic diet (Table VII, A)	Grass protein diet (Table VII, B)	Basic synthetic diet (Table VII, C)	Low cellulose diet (Table VII, D)	High cellulose synthetic diet (Table VII, E)	Basic synthetic diet + chlorophyll
Schistocerca:								
A	340	246, 459	227, 160	295, 379				
B		140, 388	258, 252	341, 427	265, 237			
C		350, 410			356, 338	380, 378		394, 380
D		305, 284		425, 413	318, 329	226, 386		348, 408
E		500,			386, 372	377, 420	350, 321	
Means	340	342	224	380	325	361	336	383

Locusta:

Exp.	Grass	Basic diet	Low cellulose diet	High cellulose diet	Basic diet without oil	Basic diet with low oil	Basic diet with double oil	Basic diet with no carotene	Basic diet with low carotene
A	360, 374	208, 209	257, 283	317, 287					
B	354, 278	329, 300			242, 251	291, 323	325, 316		
C	184, 292	274, 230						320, 229	328, 264
Means	307	258	270	302	247	307	321	275	296

are based; all diets, including grass and those with high and low proportions of cellulose, gave values in the range 247—321. With *Locusta* also, the utilisability of food appears to be of paramount importance in regulating the amount eaten.

DISCUSSION

Phagostimulant properties of substances used in artificial diets for locusts

Of various substances tested, only certain sugars, wheatgerm oil and an ether extract of bran were notable in causing hungry nymphs of *Schistocerca* to eat consistently greater amounts of filter paper than did distilled water. Most other components used in artificial diets gave negative results, although more detailed study might have revealed slight acceptant or rejectant effects. However, none were comparable with sucrose in stimulating feeding.

Somewhat anomalous results were obtained with yeast and a dried grass-juice preparation "Cerophyll", both of which are substances which improved growth on early artificial diets. Extracts of the former seemed to have both rejectant and acceptant properties, while "Cerophyll", which might have been expected to contain any phagostimulants possibly present in grass, a normal food, gave only a weakly positive response compared with sucrose, and appeared to be completely rejected at high concentration. In these circumstances, and bearing in mind that the markedly phagostimulant substances sucrose and wheatgerm oil were present in all early diets, it seemed doubtful whether poor growth could be ascribed to deficient phagostimulation.

Utilisation of complete diets by locusts in relation to amounts ingested

The question of palatability was again probed in an attempt to determine why improved growth was brought about by the inclusion of crude grass-protein in synthetic diets. An unexpected outcome of this work was the discovery that owing to a very high coefficient of utilisation, little of the synthetic diets at first in use were egested as faeces. As the superior, grass-protein diet had a lower coefficient of utilisation and gave greater faecal production, it was envisaged that poor growth might be caused by protracted mechanical difficulties in passing food deficient in inert bulk material through the gut. In the light of this hypothesis synthetic diets were reformulated so as to contain greatly increased mounts of cellulose, and were then found to support satisfactory growth.

In these ingestion experiments it was noted that increments in hopper weight during the short period of growth entailed differed but little on extremely different foods. This led to an examination of the relationship between the amount of food eaten and its utilisability, from which it became quite clear that utilisability was a major factor in regulating the amount of food eaten. The evidence suggested that within the class of satisfactory foods (i.e. grass and those synthetic diets which gave good growth in extended growth trials) the amount of *utilisable* material to be ingested was more or less constant.

Factors which affect feeding activity in insects

The implications of this are of great interest in relation to previous ideas on the nature and regulation of feeding responses in insects. Work on this general topic falls into two very closely integrated spheres. On the one hand a large body of information relates to the acceptability of solutions of various sugars by adult insects, mostly Hymenoptera, Lepidoptera and Diptera which normally feed on sugary secretions of plants. On the other hand, information has been sought on the nature and mode of action of those "token substances" which must be present in the food of mono- and oligophagous insects to initiate and sustain feeding.

Much of the early work on sugar acceptance has been reviewed by DETHIER (1953b) and the following points are relevant to the present discussion. Acceptance of sugar solutions does not necessarily depend on their nutritive value, although those most readily accepted are generally the most nutritive. Where the quantity of solution to be taken has been measured, it has been found to increase with concentration, frequently in a logarithmic way.

From a detailed study of *Phormia* it was concluded that although the absolute amount of sugar ingested increased over the whole concentration range employed, there was no relationship between the amount of sugar taken and its nutritive value; initiation and completion of the feeding reaction were completely controlled by the integrated input from chemosensory systems which responded to non-nutritive as well as nutritive sugars (DETHIER, EVANS & RHOADES, 1956). It was recognized that the effective thresholds of the sensory systems were affected by the overall nutritional state of the insect, but later work showed that changes in threshold were not directly mediated by endogenous factors (EVANS & DETHIER, 1957).

It will be seen that these mechanisms offer little indication that the state of nutrition participates directly in the regulation of feeding. They appear to constitute a means whereby insects whose normal food is largely sugar are led to concentrate their feeding whenever possible on fluids rich in their required nutrient. As such, they are perhaps best regarded as an adaptive specialisation of a readiness to eat sugar which occurs in a variety of animals of diverse feeding habit.

Amongst insects which do not normally feed on sugary fluids, sugars have been shown to elicit a feeding response in carnivorous water beetles (DETHIER, 1953b), wireworms of the spp. *Agriotes* (THORPE, CROMBIE, HILL & DARRAGH, 1947), larvae of the European corn borer *Pyrausta* (BECK, 1956a) and the Colorado beetle *Leptinotarsa* (THORSTEINSON, 1958). The occurrence of such a response in *Leptinotarsa* is of great interest, as this typical oligophagous insect has hitherto been thought to require "token" stimulants occurring in certain Solanaceous plants to release a feeding response (DE WILDE, 1958). The response of first instar larvae of *Pyrausta*, the intensity of which was shown to be logarithmically related to the concentration of sugar, was not a necessary feature of normal feeding, for larvae in this stage grew normally on diets devoid of carbohydrate (BECK, 1956b).

Locusts are among those insects which respond positively to sugars, and there was some indication that the magnitude of the response is logarithmically related to sugar concentration. Tests not included in the foregoing account showed that *Schistocerca* nymphs responded markedly to sucrose, maltose, glucose and fructose, less well to lactose, mannose and sorbose, doubtfully to galactose, and not at all to xylose. All these sugars with the exception of xylose, sorbose and possibly galactose were of nutritive value to *Schistocerca* (DADD, 1960b), and it appears that this insect conforms to the general pattern that acceptability is not necessarily correlated with nutritive value.

Tests using filter papers moistened with distilled water have shown that feeding may occur in the absence of special stimuli. Elsewhere evidence has been offered to show that third instar nymphs of *Schistocerca* at first grow normally on synthetic diets totally devoid of carbohydrates (DADD, 1960b). It therefore appears that although sugars are among the most powerfully phagostimulant substances for locusts, their importance in overall feeding behaviour when dealing with a complete diet is at most marginal. Similar considerations apply to wheatgerm oil, the only other component of synthetic diets here found to have strong phagostimulant properties.

What then regulates feeding in locusts? A state of hunger is in itself sufficient to initiate feeding, in the absence of rejectants. Should what is eaten be of balanced nutritional value and of suitable mechanical properties the evidence suggests that feeding is sustained by endogenous drives which are regulated by the utilisability of the ingested material.

The possibility of such a mechanism has recently been discussed by KENNEDY (1958) in relation to work on the ingestion of sap by aphids (MITTLER, 1954), where it was shown that *Tuberolachnus* feeding on willow stems containing sap of low nitrogen content ingested more sap and egested more honeydew than those on stems high in nitrogen. In both cases the total nitrogen ingested was closely related to the nitrogen gain of the aphid tissues. This case differed from the situation in locusts in that poor sap, even though it was taken in greater quantities, was unable to support good growth. This may have been a result of the passive nature of sap feeding in aphids (KENNEDY & MITTLER, 1953), which must limit the rate of ingestion to a function of the time spent feeding. With very high proportions of cellulose in synthetic diets, a similar limit must be reached with locusts, when even continuous feeding would fail to provide adequate nutrients.

The role of nutrients in the regulation of feeding by insects

In discussing the general theory of feeding behaviour in insects the past tendency has been to ignore the possible importance of nutrients, or at best to accord them a minor role (DETHIER, 1953a, 1954; FRAENKEL, 1953a & b; LIPKE & FRAENKEL, 1956). This situation doubtless stems from the preponderance of studies dealing with two specialised types of feeding in which gustatory responses have been clearly shown to be of primary importance. In these cases the striking effects of external gustatory stimuli have overshadowed other factors which may be implicated.

As we have seen, marked phagostimulatory responses to sugar may be detected in insects for which they form no necessary part of the total feeding activity. Moreover, the presence of suitable token substances is, alone, insufficient to ensure feeding in oligophagous insects; in the well documented case of *Plusia*, nutrients as well as specific phagostimulants are required (THORSTEINSON, 1953a). Considering the obvious advantages which would accrue from a combination of the functions of nutrient and phagostimulant, it is not surprising to find that such is the case with the polyphytophagous wireworms (THORPE *et al.* 1947), several other polyphagous species (THORSTEINSON, 1956) and the carnivorous water beetle *Dytiscus* (TINBERGEN, 1936).

Although in locusts it was only possible to demonstrate marked phagostimulatory activity for sugars and wheatgerm oil, it remains possible that more delicate methods might reveal similar although weaker properties in other dietary components. Lacking this information it may nevertheless be inferred that as optimal feeding occurs on synthetic diets in which all components are nutrients with the exception of cellulose, any external gustatory stimulation of consequence must derive from nutrients. Similar arguments apply to the now numerous insects which may be reared on wholly synthetic diets.

The endogenous drive of hunger is recognised to be the prime motivator in all feeding situations. It is of great interest to find that in locusts endogenous drives related to the intake of nutritive material appear to regulate the extent of feeding thereafter, for such an arrangement would appear to be most efficient in ensuring adequate nutrition with a wide range of foods.

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RESUME

OBSERVATIONS SUR LA GUSTATION ET L'UTILISATION DE L'ALIMENT PAR LES ACRIDIENS EN RELATION AVEC L'INTERPRÉTATION DES ÉPREUVES DE CROISSANCE AVEC DES RÉGIMES SYNTHÉTIQUES

L'effet phagostimulant des substances employées dans les régimes artificiels pour *Schistocerca* a éte évalué en comparant les quantités de matière fécale produites par des nymphes affamées auxquelles étaient offertes du papier filtre trempé dans de l'eau ou dans des solutions des substances à expérimenter. Le sucrose, le glucose, l'huile de germe de blé et un extrait éthéré de son ont occasioné une excrétion nettement plus grande que celle produite par l'eau distillée seule. La chlorophylle, le carotène, la caséine, la peptone, l'huile d'olive, un mélange salin, un mélange de vitamines du complexe B, le chlorure de choline et l'acide ascorbique n'étaient pas plus efficaces que l'eau. Des extraits aqueux de levure étaient en général aussi efficaces que le sucre, mais quelquefois semblaient inhibiteurs à des concentrations fortes. Le „Cerophyll", une spécialité de jus d'herbe qui a amélioré la croissance quand on l'a ajouté à certains régimes artificiels, semblait répulsif à des concentrations fortes et moins efficace que le sucre une fois dilué. Comme les régimes qui manquaient de sucre, de levure ou d'huile de germe de blé, les seules parties constituantes des régimes synthétiques à manifester des effets nettement phagostimulants, étaient au début capables d'entretenir une bonne croissance, il paraît probable que la faim est plus importante que la présence de stimuli gustatifs spéciaux pour déclencher l'activité alimentaire. On a comparé les quantités de nourriture mangée et les faeces produits par des nymphes du 5ème stade de *Schistocerca* auquelles étaient offertes des régimes artificiels divers ou de l'herbe fraîche. La première intention était de déterminer si un régime synthétique contenant de la protéine non

raffinée d'herbe, qu'on avait trouvée supérieure dans des expériences de croissance à un régime analogue qui manquait de la protéine d'herbe, devait sa supériorité à un goût amélioré. On n'a pas découvert de différences importantes au point de vue de l'alimentation dues à des différences de goût, mais on a trouvé que les quantités de nourriture prises étaient liées à des différences considérables „d'utilisation digestive". En ce qui concerne le poids sec, l'herbe n'a été utilisée qu'à moitié aussi bien que le régime synthétique tandis que le régime „herbe protéine" était intermédiaire à cet égard. Ceci a suggéré que la meilleure croissance effectuée en incorporant l'herbe protéine, pouvait se relier avec la diminution d'utilisation. En reformulant un régime synthétique contenant davantage de cellulose pour donner une utilisation digestive de l'ordre trouvé pour l'herbe, on a obtenu une croissance aussi bonne que celle du régime „herbe-protéine". Le travail subséquent avec *Schistocerca* et *Locusta* a montré que l'utilisation digestive était un élément important qui réglait la quantité de nourriture prise. En général, plus l'utilisation était basse, plus la quantité mangée augmentait, et pour des variétés de régimes de digestibilité différente, y compris l'herbe, les valeurs obtenues en multipliant la quantité mangée (poids sec) par le coefficient d'utilisation étaient semblables pour chaque espèce. Afin d'expliquer ces résultats, il est proposé que des stimuli spéciaux gustatifs ne sont pas nécessaires au déclenchement de l'activité alimentaire chez les nymphes des criquets pélerins et qu'ils sont d'une petite importance dans la sustentation de l'alimentation. Il paraît que la quantité de nourriture prise est en grande partie réglée par son utilisation digestive en ce qui concerne une nourriture d'une composition plus ou moins suffisante, situation qui n'est pas étonnante chez des insectes qui sont capables de se développer sur des plantes diverses.

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FOOD PLANT RELATIONSHIPS OF THE DIAMOND-BACK MOTH
(PLUTELLA MACULIPENNIS (CURT.))
II. Sensory Regulation of Oviposition of the Adult Female

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Oviposition responses to natural and artificial substrates were investigated. More eggs were deposited on plants that contain isothiocyanates (natural hosts). Depletion of isothiocyanate content by sulfur-deficient plant nutrition appears to reduce attractiveness of host plants as egg substrates. Eggs are laid preferentially in concavities of leaves or stems. Significantly more eggs are laid on dimpled than smooth polyvinyl plastic surfaces. On both types of plastic substrate more eggs are laid if allyl isothiocyanate or mustard leaf juice is added. Mustard leaf juice contains allyl isothiocyanate but is more stimulating to oviposition indicating that other factors contribute to the total effect.

Although the coumarin in *Melilotus* sp. tends to inhibit oviposition, *Plutella* may be induced to increase egg deposition on such plants by treating them with allyl isothiocyanate. Unknown inhibitory constituents in tomato leaves prevent egg laying even in the presence of allyl isothiocyanate. It was concluded that oviposition is regulated significantly by external stimuli and that inhibitory chemical influences as well as positive chemical stimuli and texture of the surface are all significant factors.

The chemical influence of plant constituents on acceptance or rejection of plants as food by the larva of the diamond-back moth, *Plutella maculipennis* (Curt.) has been considered previously (GUPTA & THORSTEINSON, 1960; THORSTEINSON, 1953). The mechanisms of food plant specificity as expressed by this insect in nature must also take into account the regulation of oviposition behaviour of the adult female inasmuch as this determines what plant the newly hatched larva must accept or reject. The object of this study is, therefore, to determine what attributes of the plant are most significant as oviposition stimulants and to investigate interactions among these influences.

MATERIALS AND METHODS

Adults for the experiments were taken from a culture that had been maintained in our laboratory for three years. Newly emerged adults were isolated in new wooden cages or paper cartons provided with brass wire net for ventilation. The insects were fed on sugar solution. Plants of *Brassica juncea* (Mustard), *Lycopersicon esculentum* (Tomato), *Pisum sativum* (Garden Pea), *Melilotus officinalis* (Sweet Clover) and *Melilotus alba* (var. Coumino) used in various experiments were grown in the green house. Allyl isothiocyanate (Eastman Organic Chemicals)

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and coumarin (Fisher Scientific Ltd.) were used in various dilutions in methanol or distilled water. Glass vials with plastic caps 3 cm diameter were used in most of the experiments as an oviposition substrate. The caps had either a rugose or smooth outer surface. A hole 7 mm diameter was cut in the centre of each cap and stuffed with loose cotton. The vials were filled about two thirds with water to provide humidity in the cage although it was not established that this was necessary.

OBSERVATIONS

The moths began to lay eggs on leaves of a mustard plant during the night of the second day after emergence. The pre-oviposition period was delayed till the third or fourth day in the absence of a natural host plant. However, in a few experiments the addition of a low concentration (10 p.p.m.) allyl isothiocyanate to the rugose plastic cap seemed to affect the pre-oviposition period in that eggs were layed on the second night as occurs on the mustard plant.

On rugose plastic caps, the maximum number of eggs are laid on the ninth or tenth day after emergence and the presence of allyl isothiocyanate does not appreciably alter the course of oviposition apart from tending to shorten the pre-oviposition period as mentioned previously. The mean numbers of eggs laid by eight pairs of moths in each of two experiments are 109 and 108. This lies within the range reported by HARCOURT (1957) who recorded oviposition on cabbage. Under the conditions of his observations there was no pre-oviposition period and peak egg deposition occurred on the first night.

REGULATION OF OVIPOSITION ON A NATURAL HOST

Preliminary oviposition experiments indicated that the adult females lay more eggs on plant species that contain mustard oils. Data illustrating this are presented in Table I. The moths laid a relatively overwhelming number of eggs on cabbage (Crucifereae). *Nasturtium* (Tropaeolaceae) was appreciably less attractive. We do not yet know whether this is attributable to concentration or species of mustard oil. To the human sense the essential oil in nasturtium is much more pungent than that of cabbage.

TABLE I

Mean Number of Eggs Laid on Plant Species by P. maculipennis

Pea	Bean	Marigold	Nasturtium	Cabbage
85	52	4	370	817

No. of Replicates = 3

The two legumes, pea and bean, were much less attractive than nasturtium but apparently these contain only weak inhibitors of oviposition if any. The composite, marigold, was least attractive, possibly suggesting the operation of inhibitory influences as well as a deficiency in stimulants. It is interesting that legumes partially satisfy the stimulus requirements for oviposition since they approach acceptability also as food for the larvae (GUPTA & THORSTEINSON, 1960).

Influence of Mineral Nutrition of Plant

The fact that mustard oils are sulphur compounds suggested that mustard plants starved of sulphur would contain much less free mustard oil, a state that might render them less attractive for oviposition. White mustard plants (*Brassica alba*) were cultured hydroponically in a complete nutrient solution. A parallel series of plants was cultured in nutrient solutions deficient only in sulphur. Analytical quality reagents were used throughout. Young plants at the four or five leaf stage were used in the experiments. The sulphur present in the seed appeared to supply the essential requirements of the plants up to this stage, since no deficiency symptoms were in evidence, yet no trace of mustard oil could be detected by human taste in leaves from the sulphur-restricted plants.

In thirteen such tests the mean number of eggs deposited on plants grown in a full nutrient solution exceeded those laid on the sulphur-restricted plants (mean difference 14.1), but the data variability precluded any conclusive inference. The mustard oil in *B. alba* is oxybenzyl isothiocyanate and to the human sense is odourless.

The experiments were repeated with black mustard (*B. nigra*). This species contains allyl isothiocyanate which is strongly odourous to the human sense. In the five replicates, more than twice as many eggs were laid on plants grown in a full nutrient solution than on the plants deprived of sulphur (mean difference 95.0). As the standard error is 46.6 the difference falls short of the 5% level of significance but seems to be suggestive. A more interesting comparison could be made if mustard plants genetically devoid of mustard oils were available comparable to Coumino clover which lacks coumarin, the odour constituent in other sweet clover varieties.

INFLUENCE OF EGG SUBSTRATE SURFACE

Moths generally laid eggs on glass, paraffin wax, earthenware pot, plastic vial, plastic cap but rarely on paper, brass or saran screen. Our experiments with plastic caps clearly indicate that the majority of eggs are laid on those having a rugose surface both in the presence or absence of a favourable chemical stimulus (Table II) provided no inhibitors are present. The fact that moths prefer to lay eggs on rugose rather than on a smooth surface is further supported by our experiments (*vide infra*) using common sweet clover, coumino sweet clover, pea and tomato plants. On sweet clover, coumino clover and pea which bear smooth leaves, the eggs were seldom deposited on the leaf but were generally laid in the axils of

TABLE II

Average number of eggs on the two types of plastic caps

Kind of Treatment	Number of Eggs		Difference
	Rugose cap	Smooth cap	
1. No treatment	44.9	10.6	34.3 **
2. Allyl isothiocyanate	51.4	10.6	40.8 **
3. Juice from fresh mustard leaf — "light" dose	40.25	28.5	11.75**

Replicates — 8

Note: Experimental design does not provide for comparisons between chemical treatments.

** Difference significant at 1% level

leaves, the groove in the pedicel and cut surfaces on the stem. On the other hand most of the eggs laid on the tomato plant were confined to the rugose leaf surface.

INFLUENCE OF CHEMICAL CONSTITUTION OF MUSTARD LEAVES

The expressed juice of *B. juncea* leaf was painted on the outer surface of a smooth cap. One freshly treated cap and another untreated were introduced every evening into a cage containing a number of moths. Next morning the numbers of eggs on the caps were counted. The results of ten replicates are given in Table III. On the first day, more eggs were found on the untreated than on the treated cap. But when the same pair of caps had been left in the cage for another 24 hours, appreciably more eggs were deposited on the treated than on the untreated cap. Both of these effects are statistically significant. It appears that the juice exerted an inhibitory influence on egg deposition during the first night, but stimulated oviposition on the second night. This reversal is understandable because trituration of the leaves exposes the glucosides to the hydrolytic action of myrosin (an enzyme localized in separate cells in the intact plant). The hydrolysis results in a considerable release of free mustard oil which may exceed the optimal concentration. It is suggested that losses through evaporation or otherwise reduce the concentration of mustard oil to a favourable level after about 24 hours. In later experiments the amount of juice from fresh mustard leaf applied on the cap was gradually reduced until the egg count on the first morning indicated that the juice concentration was optimal (Table III). Similar dosages were applied to the plastic caps in further experiments.

TABLE III

Average Number of Eggs Laid on Smooth and Rugose Caps Treated with Juice From Fresh Mustard Leaf

Time after application	Heavy Dose			Light Dose					
	Smooth cap			Smooth cap			Rugose cap		
	Number of Eggs		Difference	Number of Eggs		Difference	Number of Eggs		Difference
	On treated cap	On untreated cap		On treated cap	On untreated cap		On treated cap	On untreated cap	
Same night	2.8	14.5	11.7**	25.25	3.75	21.50**	32.00	8.20	23.8**
Next night	22.4	2.9	19.5**	23,75	1.50	22.25**			

No. of Replicates in all tests — 10.
** Difference significant at 1% level

The results from experiments with the juice of fresh mustard leaf suggested the possibility that mustard oil, a strongly odoriferous substance obtained as a fission product of mustard oil glucosides present in cruciferous plants, may contribute a significant stimulus that regulates host plant specificity for oviposition in *Plutella*. A common representative of the mustard oils, allyl isothiocyanate, available in synthetic form, was selected for investigation.

In order to establish an optimal dosage, various dilutions of allyl isothiocyanate in uniform volumes were applied to rugose plastic caps. The egg counts presented in Table IV indicate that allyl isothiocyanate is repellent at 1000 p.p.m. but a

TABLE IV

Average Number of Eggs on Rugose Plastic Vials Treated With Uniform Volumes of Allyl Isothiocyanate in Different Dilutions

Dosages of Allyl-isothiocyanate	Number of Eggs		
	Treated cap	Untreated cap	Difference
1000 p.p.m.	5.2	13.8	8.6*
100 p.p.m.	11.6	24.2	12.6
10 p.p.m.	19.8	3.0	16.8**
1 p.p.m.	5.2	8.6	3.4

No. of Replicates = 6

* Difference significant at 5% level

** Difference significant at 1% level

dilution of 10 p.p.m. is a stimulant for oviposition. In further experiments allyl isothiocyanate at this optimal strength was used.

Although allyl isothiocyanate at an optimal dosage is clearly an ovipositional stimulant, the existence of other chemical stimulants in the leaves of the host plant is not precluded. Rugose plastic caps treated with mustard leaf juice were compared with similar caps treated with allyl isothiocyanate (10 p.p.m.) in seven replicated pairs. The mean number of eggs laid on plastic caps treated with mustard leaf juice was 85 compared with a mean of 16 on caps treated with allyl isothiocyanate. The mean difference, 69, is significant at the 1% level. It is inferred that mustard leaves contain constituents that enhance the ovipositional stimulation of allyl isothiocyanate.

Interaction of Physical and Chemical Stimuli

As has been demonstrated (see Tables II and III), both surface and odour are individually involved in the regulation of oviposition. The possible interaction of these two stimuli was investigated in two series of simple factorial experiments. Four plastic vial caps served as oviposition substrates. Two had a rugose surface and the other two were smooth. One rugose and one smooth cap were treated with a uniform volume of allyl isothiocyanate (10 p.p.m.), the other two caps were untreated. The four caps were presented to a group of caged moths in the

TABLE V

Combined Influence of Surface and Odour by Oviposition in P. maculipennis

Treatment				Mean No. of Eggs	
Surface		Odour		Odour of mustard leaf juice	Odour of 10 p.p.m. allyl isothiocyanate
Smooth	Rugose	Present	Absent		
	†	†		89.3	56.3
	†		†	15.8	23.0
†		†		68.0	8.0
†			†	2.5	4.8
				Interaction*	Treatments**

No. of replicates = 6

Factorial Analysis based on transformation $\sqrt{x + 0.5}$

* Interaction significant at 5% level

** Treatment means differ significantly at 1% level evening and the number of eggs next morning was recorded. This experiment was repeated, substituting allyl isothiocyanate by an application of an optimal dose of mustard juice. The data presented in Table V show that positive tactile and chemosensory stimuli induce more oviposition than either stimulus alone. The effect of mustard leaf juice, however, is relatively much greater on a smooth than on a rough surface (interaction).

OVIPOSITION ON PLANTS OUTSIDE NATURAL HOST RANGE

In the studies of food plant specificity of the larvae, considered in the previous paper (Gupta & Thorsteinson, 1960) the importance of investigating the behavioural relationships toward "foreign" food plants was emphasized. The same principle applies to the study of the adult in its food plant relationships. Inasmuch as *Plutella* larvae accept certain legumes (pea, sweet clover, coumino clover) more readily than other foreign plants it was of special interest to study the oviposition behaviour of the adults on these plants. As an example of a plant not at all acceptable to the larvae even when sinigrin is supplied, the tomato plant was selected. Oviposition was tested on whole plants, plant cuttings and expressed juices applied to artificial surfaces.

Oviposition on Whole Plants

A small potted plant was placed in each of two cages, both containing approximately the same number of moths. One plant was treated with allyl isothiocyanate in dilution of 10 p.p.m. before being introduced into the cage in the evening. The other plant was not treated. The number of eggs on plants and pot was recorded the following morning. As far as possible, new plants were used every day. The suitability of the plants as oviposition substrates was estimated by comparing the number of eggs laid on the plants with the number of eggs laid on the corresponding pots. The data are presented in Table VI.

TABLE VI

Effect of Allyl Isothiocyanate on Oviposition by P. maculipennis *on Non-Host Plants*

	P. sativum		*M. officinalis*		*M. alba* (var. Coumino)		*Lycopersicum* sp.	
Treatment	Plant	Pot	Plant	Pot	Plant	Pot	Plant	Pot
Untreated	14.4	18.4	0.6	16.8	12.6	2.0	3.7	24.67
Difference	4.0		16.2**		10.6**		20.7*	
Allyl Isothiocyanate (10 p.p.m.)	2.0	41.24	14.4	0.2	23.5	0.28	14.67	14.3
Difference	30.7**		14.2**		23.2**		0.4	

Number of replicates = 6

* Difference significant at 5% level

** Difference significant at 1% level

Anomalous results were obtained with *P. sativum* in that the treated plant is a relatively less suitable oviposition site than the pot whereas oviposition on the untreated plant is approximately the same as on the pot. The two *Melilotus* species provide a contrast in that the untreated *M. officinalis* plant (naturally containing coumarin) is less favourable for oviposition relative to the pot whereas oviposition on *M. alba* var. Coumino (devoid of coumarin) significantly exceeds that on the pot. When either of these species is treated with allyl isothiocyanate, oviposition on the plant greatly exceeds that on the pot. This implies that coumarin tends to inhibit oviposition but allyl isothiocyanate masks this effect. The untreated tomato plant apparently presents some inhibitory influence which seems to be only partially overcome by treatment with allyl isothiocyanate. The inhibitor in tomato is evidently more decisive than coumarin in spite of the favourable rugose surface of the tomato leaf as compared with smooth *Melilotus* leaves.

Oviposition on Plant Cuttings

Cuttings of the same plant species were tested in the same way as the entire plants except that the stalks were immersed in vials containing water. The oviposition data are presented in Table VII. It will be noticed that the results differ somewhat from those obtained with entire plants in pots. This might be a result of some unknown effect of excision *per se* on the attractiveness or repellency of the plant cuttings, but there is no evidence of this. Alternatively, the relative areas and mean distances between the plant surfaces and the pot or vial or the reduced number of crevices on the plants might have modified the oviposition responses. However some of the results obtained previously were confirmed. The pea plant cutting is less attractive than the glass vial in which it is supported whereas the entire plant (Table VI) competed successfully with the pot. As in the entire plant experiments, treatment with allyl isothiocyanate does not increase the attractiveness of the pea plant cutting. The results obtained with *M. officinalis* were essentially the same with entire plants and cuttings. Coumino clover cuttings and vials favoured egg deposition equally whether allyl isothiocyanate was applied to the leaves or not. Tomato cuttings were more attractive than the vials and this preference was increased by treatment of the cuttings with allyl isothiocyanate.

TABLE VII

Influence of Allyl Isothiocyanate on Oviposition by P. maculipennis *on Plant Cuttings*

eat- nt	*Pisum sativum*			*Melilotus officinalis*			*Melilotus alba* (var. Coumino)			*Lycopersicum esculentum*		
	Plant	Vial	Diff.	Plant	Vial	Diff.	Plant	Vial	Diff.	Plant	Vial	Diff.
ɔ at- ent	1.00	40.75	39.75**	6.91	29.67	22.76**	21.0	29.0	8.0	8.75	2.65	6.13*
lyl othio- anate	3.2	34.6	31.44**	21.67	15.0	6.67*	29.0	27.5	1.5	35.75	4.25	31.50*

Number of replicates = 10
* Difference significant at 5% level
** Difference significant at 1% level

The inhibitory influence demonstrated in the experiments with entire tomato plants was not manifested by tomato cuttings.

Juice from fresh leaves

Juice from fresh leaves of each species of the plants was applied to rugose plastic caps in the evening. Two vials containing water, one closed with one of the treated caps and the other with an untreated rugose cap, were introduced to moths in a paper carton cage. Similar sets of vials were treated with a measured quantity of allyl isothiocyanate (10 p.p.m.) and were tested as oviposition substrates in the same way. The number of eggs laid on the caps was recorded in the morning. The egg deposition responses to juice of tomato and sweet clover leaves are presented in Table VIII. Both treatments are unfavourable to oviposition by *P. maculipennis* and the failure of allyl isothiocyanate to increase egg deposition in this experiment indicates that the juices of tomato and sweet clover contain substances that inhibit oviposition.

TABLE VIII

Influence of Expressed Juice of Non-Host Plants and Allyl Isothiocyanate on Oviposition of P. maculipennis *on Plastic Caps*

Source of Plant Juice	Allyl Isothiocyanate (10 p.p.m.)	Caps Treated with Plant Juice	No Plant Juice	Difference
Melilotus officinalis	Not added	1.2	12.8	11.6*
	Added	1.3	33.2	31.9*
Lycopersicum esculentum	Not added	0.2	6.0	5.8**
	Added	0.5	7.8	6.3**

Number of replicates = 10

* Difference significant at 5% level

** Difference significant at 1% level

EFFECT OF COUMARIN

Preliminary experiments were conducted to determine whether coumarin present in common sweet clover inhibits oviposition. Alcoholic solutions of coumarin in dilutions of 25 and 100 p.p.m. were applied to the leaves of two Coumino sweet clover cuttings (naturally coumarin-free) inserted in vials containing water and closed with smooth caps. The vials were introduced into separate paper carton cages and the number of eggs recorded next morning. The results of the test indicated that coumarin at these dilutions does not favour oviposition since most of the eggs were laid on the vial and the cap rather than on the plant. On the other hand, in a variation of these experiments wherein the vial and the relief cap were treated with coumarin instead of the leaves, practically all the eggs were laid on the plant.

The inhibition of oviposition on rugose plastic caps by lower concentrations of coumarin (1—25 p.p.m.) was investigated. The results, presented in Table IX, indicate that 25 p.p.m. coumarin approximates the threshold concentration for inhibition under the conditions of the experiment.

In other experiments, however, where 25 p.p.m. coumarin solution was applied

TABLE IX

The Influence of Coumarin on Oviposition by P. maculipennis *on a Rugose Plastic Surface*

Dilution of coumarin in parts per million	Mean Numbers of Eggs Laid Treated	Untreated	Difference
1	48	52	4
5	56.5	59.97	3.47
25	44.5	80.0	35.5**

Number of replicates = 8

** Difference significant at 1% level

to mustard leaves (*B. juncea*), most of the eggs were deposited on the leaves rather than the vial and cap. Apparently the oviposition stimulants in this host plant tend to countermand the inhibitory influence of coumarin.

DISCUSSION

It has been established in this investigation that an optimal concentration of allyl isothiocyanate, one of the mustard oils characteristic of the food plants of *P. maculipennis,* provides a stimulus that accelerates or otherwise favours oviposition by this insect. This fact contributes to our understanding of food plant specificity of *P. maculipennis* but does not wholly account for it in view of the demonstration that this odour stimulus manifests itself conspicuously only on a favourable (rugose) surface and in the presence of other unknown, presumably odourous, constituents of the food plants. Moreover, a favourable surface elicits considerable egg deposition even in the apparent absence of any stimulating odour. It is significant also that at least one plant (Coumino sweet clover) devoid of both mustard oils and odourous inhibitors provides a moderately good egg substrate even though its leaves are smooth and do not provide the optimal surface texture. Another variety of sweet clover (*M. officinalis*) is less favourable for egg deposition apparently because of the coumarin content. It is difficult to avoid the conclusion that the unattractiveness of non-host plants is not conditioned solely by the presumed absence of odourous oviposition stimulants but is significantly regulated by the occurrence of chemosensory inhibitors as well as by the surface texture of the leaves. In this regard we must invoke inhibitory as well as stimulatory chemosensory stimuli in order to understand host plant specificity in regard to oviposition as we did in the study of feeding behaviour of the larvae (GUPTA & THORSTEINSON, 1960).

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RESUME

Nous avons étudié la ponte au point de vue des réponses de *Plutella maculipennis* aux surfaces naturelles et artificielles. La ponte s'est avérée plus forte sur les plantes qui contenaient des isothiocyanates (hôtes naturels). La baisse du contenu d'isothiocyanate dans une nourriture pauvre en souffre semble réduire l'attraction de la surface des hôtes à la ponte. L'insecte préfère pondre ses oeufs dans les concavités des feuilles ou des tiges. D'une manière significative, la ponte s'avère plus forte sur une surface rugueuse que sur des surfaces lisses de plastiques polyvinyls. La ponte est plus élevée sur la surface des deux espèces de plastiques si on ajoute du jus de feuilles de moutarde ou de l'allylisothiocyanate. L'allylisothiocyanate est un des constituants de jus de la feuille de moutarde, mais exerce une action moins prononcée sur la ponte que ce jus. Ceci indique que d'autres agents contribuent à l'effet total.

Bienque la coumarine dans *Melilotus* (sp.) ait tendance à inhiber la ponte, on peut induire *Plutella* à augmenter la ponte sur de telles plantes en les traitant avec de l'allylisothiocyanate. Certains constituants inhibitifs inconnus qu'on trouve dans les feuilles de *Lycopersicum* empêchent la ponte même avec traitement d'allylisothiocyanate. La conclusion s'impose: la ponte est fortement influencée par des excitants externes et les inhibitions causées par des influences chimiques aussi bien que par des excitants chimiques favorables, de même que la texture de la surface en question. Ces choses sont tous des facteurs importants.

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EFFETS COMPARES DE JEUNES INTERMITTENTS ET DE PERIODES EQUIVALENTES DE SUBSISTANCE SUR DES VARIETES RESISTANTES OU SENSIBLES DE POIS, *PISUM SATIVUM* L., SUR LA CROISSANCE, LA REPRODUCTION ET L'EXCRETION DU PUCERON DU POIS, *ACYRTHOSIPHON PISUM* (HARR.) (HOMOPTERES: APHIDIDES)

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La croissance et la reproduction du puceron du pois, *Acyrthosiphon pisum* (Harr.), soumis à des jeûnes intermittents ou à des périodes équivalentes de subsistance sur des plantes-hôtes résistantes, (*Pisum sativum* L., variétés Onward et Melting Sugar), ont été réduites proportionnellement à la durée des traitements. Les larves élevées continuellement sur l'hôte résistant ont atteint un poids moyen équivalent à celui des larves qui jeûnèrent de 10 à 12 hr par jour; celles qui furent placées quotidiennement sur l'hôte résistant de 8 à 14 hr ont atteint un poids moyen équivalent au poids de celles qui jeûnèrent de 4 à 8 hr par jour, le reste du temps étant passé sur un hôte sensible (variété Perfection). La fréquence et le taux d'excrétion des pucerons replacés sur un hôte sensible après un jeûne ont été doublés ou davantage, comparativement aux témoins. Cependant, aucune augmentation dans l'excrétion n'a été observée lorsque les pucerons à la suite d'un jeûne étaient replacés sur un hôte résistant. Ces résultats suggèrent que l'ingestion de la sève par l'insecte peut être accélérée au besoin sur un hôte sensible, mais ne peut pas l'être sur un hôte résistant.

Des travaux antérieurs sur la résistance des variétés de pois, *Pisum sativum*, au puceron du pois, *Acyrthosiphon pisum*, ont indiqué que certains facteurs de résistance semblent étroitement liés à la nutrition des aphides. Ainsi, MALTAIS & AUCLAIR (1957) ont démontré que le rapport glucides-matières azotées des plantes-hôtes était de 23.4 à 63.7 p. 100 plus élevé chez les variétés résistantes Laurier, Champion of England et Melting Sugar que chez les variétés sensibles. De plus, AUCLAIR, MALTAIS & CARTIER (1957) ont rapporté que la teneur en amides, en acides aminés et en protéines de variétés sensibles telles que Perfection et Lincoln était plus élevée que celle des variétés résistantes. Il a été aussi établi que les taux d'ingestion et d'excrétion des aphides (AUCLAIR 1958b, 1959), leur accroissement pondéral et leur reproduction (CARTIER 1959, et travaux non publiés), étaient significativement plus élevés sur des hôtes sensibles (e.g. Perfection) que sur des hôtes résistants (e.g. Melting Sugar et Onward). Toutes ces données expérimentales semblent donc indiquer que sur un pois résistant les pucerons s'alimentent insuffisamment pour maintenir une croissance et une reproduction optimales. En vue de confirmer cette hypothèse, des travaux furent effectués pour comparer les effets que peuvent avoir sur la croissance, la reproduction et l'excrétion du puceron du pois, des périodes de jeûne et de subsistance sur des variétés résistantes ou sensibles. Des résultats préliminaires de ces travaux ont été rapportés par AUCLAIR & CARTIER (1960).

METHODES

Les conditions de la serre, la culture des pois, et la façon de mesurer la fréquence d'excrétion des aphides et la densité optique des taches de miellat ont été décrites par AUCLAIR (1958a). Des virginipares aptères de la lignée R1 (CARTIER 1959), nées dans un même intervalle de 24 hr, furent utilisées à partir du deuxième ou troisième âge jusqu'au stade adulte. Durant les périodes d'alimentation, les pucerons étaient confinés à la partie supérieure des plants sensibles (Perfection) ou résistants (Onward ou Melting Sugar) jusqu'au niveau du quatrième entrenoeud, la pousse terminale représentant le premier entrenoeud. A la fin des expériences, les aphides étaient pesés par groupe de dix sur une balance de précision „Gramatic'' d'une sensibilité de 20 microgrammes.

Dans une autre expérience, le volume individuel de 350 gouttelettes de miellat fraîchement excrétées, et provenant d'une population d'environ 300 larves du même âge et en croissance du premier stade larvaire au stade adulte, fut mesuré avec une micropipette calibrée au 0.02 de microlitre et selon une technique déjà décrite (AUCLAIR 1958a). De plus, le diamètre individuel des taches formées sur les papiers filtres par 650 autres gouttelettes semblables fut déterminé à 0.16 de millimètre près, avec un micromètre oculaire calibré et sous un grossissement de 45 fois. Car, dans des conditions déterminées, les surfaces des taches produites sur papier filtre par des gouttelettes colorées sont proportionnelles exponentiellement aux volumes correspondants (BUCK, KEISTER & ZELLE 1950). Une courbe préparée des données diamètres (ou surfaces) versus volumes a donc été utilisée dans les expériences 3, 4 et 5 pour estimer le volume des gouttelettes par le diamètre des taches qu'elles formaient sur les papiers filtres. Dans le texte qui suit, la *fréquence* indique le nombre de gouttelettes (ou de taches) et le *taux* indique le volume (en microlitres) de miellat excrété durant une période donnée. Le taux est évidemment obtenu en multipliant la fréquence par le volume moyen des gouttelettes pour une même période.

Mesure des effects de jeûnes intermittents comparés à ceux de périodes équivalentes de subsistance sur des variétés résistantes

Des groupes de dix aphides chacun furent élevés en quadruplicata à raison d'un groupe par plant sur la variété Perfection, sauf durant des périodes quotidiennes de 2 à 16 hr de jeûne *ou* de subsistance sur l'une des variétés Onward et Melting Sugar. Durant le jeûne, les insectes étaient placés dans des boîtes de Pétri dont les parois intérieures étaient tapissées de papier filtre (Whatman no. 1) afin d'en recueillir le miellat. Ces papiers, de même que ceux placés sous les plants infestés, étaient ensuite traités à la ninhydrine afin de révéler la présence et le nombre de taches de miellat. Lorsque le miellat des pucerons jeûnants n'était pas recueilli, le fond des boîtes était recouvert d'un papier filtre humide. Les groupes témoins demeurant sur la variété sensible ou résistante n'étaient déplacés que durant quelques minutes, deux fois par jour, afin qu'ils subissent la même manipulation que les autres groupes. Au début des expériences, les plants étaient âgés de 18 jours, avaient six entrenoeuds et mesuraient six pouces de hauteur en moyenne.

Protocole expérimental: — L'expérience 1 comprenait sept lots de quatre groupes de pucerons répartis comme suit: un lot témoin (P) placé sur quatre plants Perfection; un deuxième lot témoin (M) placé sur quatre plants Melting Sugar; et cinq lots traités comme (P) mais qui subirent de plus un jeûne quotidien de 4 et 12 hr (4J et 12J), *ou* une période quotidienne de subsistance de 4, 12 et 16 hr sur des plants Melting Sugar (4M, 12M et 16M).

L'expérience 2 comprenait huit lots répartis comme suit: P, 8J, 12J, 16J, et, sur la variété Onward, N, 8N, 12N et 16N.

L'expérience 3 comprenait onze lots répartis comme suit: P, 2J, 4J, 8J, 12J, et, sur la variété Onward, N, 2N, 4N, 8N, 12N et 16N. Les pucerons des lots 8J et 12J jeûnèrent quotidiennement de 9 a.m. à 5 p.m. et de 9.30 a.m. à 9.30 p.m. respectivement, tandis que ceux des lots 8N et 12N furent transférés sur les plants Onward durant ces mêmes périodes. La fréquence d'excrétion et le diamètre moyen des taches de miellat furent mesurés chez les lots P, 8J, 12J, N, 8N et 12N durant quatre périodes de 12 hr (A, B, C, D) séparées par un intervalle de 48 hr entre B et C. Le taux de reproduction fut aussi déterminé durant les 48 hr qui suivirent la pesée des pucerons adultes. Durant cette dernière période, les pucerons demeurèrent continuellement sur les plants.

Mesure des effets de jeûnes intermittents sur l'excrétion subséquente des pucerons placés sur une variété résistante ou sensible

Des groupes de dix pucerons chacun furent élevés à raison d'un groupe par plant sur les variétés Perfection ou Onward, sauf durant des périodes quotidiennes de 8 ou 16 hr de jeûne dans les boîtes de Pétri. Le traitement des groupes témoins et des aphides jeûnants ainsi que la détermination de la fréquence d'excrétion et du poids final des pucerons ont été faits tel que décrit précédemment. Au début des expériences 4 et 5, les plants étaient âgés de 20 et 24 jours, avaient en moyenne 6.5 et 7.5 entrenoeuds et mesuraient 5 et 8 pouces de hauteur en moyenne.

Protocole expérimental: — L'expérience 4 comprenait six lots de quatre groupes de pucerons répartis comme suit: deux lots témoins (P et N) placés respectivement sur quatre plants Perfection et quatre Onward; quatre lots (P8J, P16J, N8J, N16J) placés sur Perfection ou Onward à l'exception d'un jeûne quotidien de 8 ou 16 hr, de 8.30 a.m. à 4.30 p.m. ou de 4.30 p.m. à 8.30 a.m. La fréquence d'excrétion et le diamètre moyen des taches de miellat furent calculés pour une période de 72 hr, et la densité optique des taches sur les papiers fut mesurée (AUCLAIR 1958a) pour une période de 24 hr.

L'expérience 5 comprenait deux lots témoins (P et N) de huit groupes de pucerons chacun, placés respectivement sur huit plants Perfection et huit Onward, et deux lots (P8J et N8J) de huit groupes chacun placés *alternativement* sur Perfection et Onward, à l'exception d'un jeûne quotidien de 8.30 a.m. à 4.30 p.m. Par exemple, les pucerons du lot P8J ont jeûné de 8.30 a.m. à 4.30 p.m., ont été placés sur huit plants Perfection de 4.30 p.m. à 8.30 a.m., ont jeûné de nouveau jusqu'à 4.30 p.m., ont été placés ensuite sur huit plants Onward de 4.30 p.m. à

8.30 a.m., et ainsi de suite jusqu'à maturité. L'excrétion fut mesurée selon l'expérience 4.

DISCUSSION DES RESULTATS

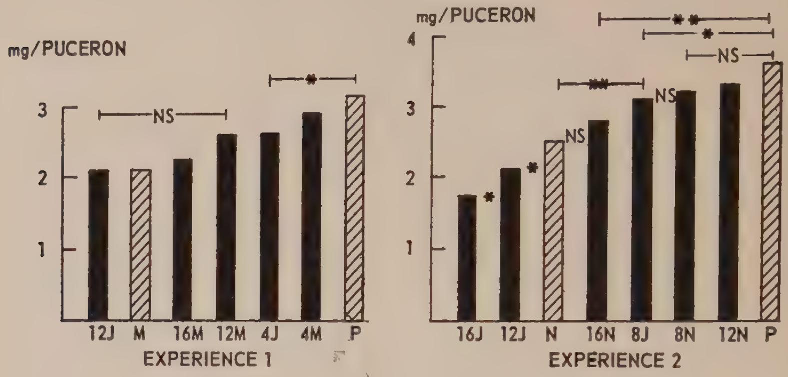

Fig. 1. Poids final moyen (mg) de pucerons (*Acyrthosiphon pisum*) élevés sur la variété sensible Perfection, mais exposés à des périodes quotidiennes de 4 à 16 hr de jeûne (4J à 16J) ou de subsistance sur une des variétés résistantes Melting Sugar et Onward (4M à 16N), comparativement à des témoins élevés continuellement sur les variétés sensible (P) ou résistantes (M ou N). Différences non significatives (NS) ou significatives à cinq (*) ou un (**) p. 100.

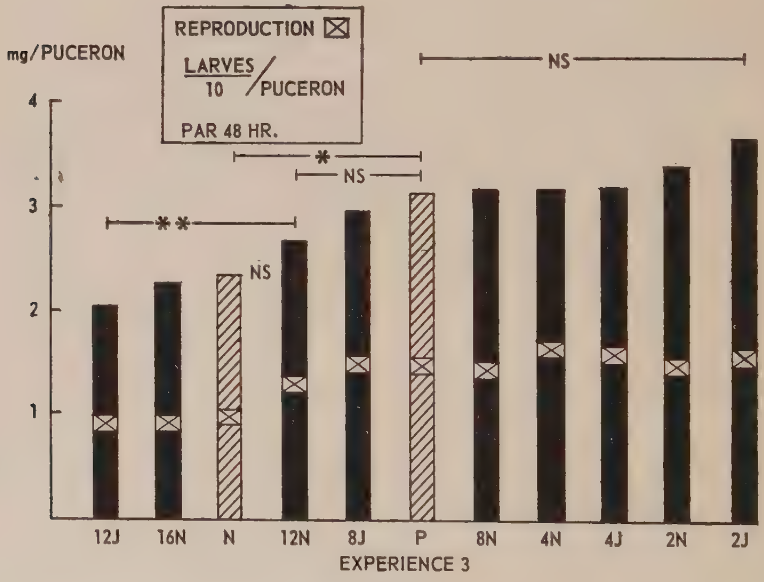

Fig. 2. Poids final moyen (mg) et reproduction ($\frac{\text{larves}}{10}$ par puceron par 48 hr) de pucerons (*Acyrthosiphon pisum*) élevés sur la variété sensible Perfection, mais exposés à des périodes quotidiennes de 2 à 12 hr jeûne (2J à 12J) ou de 2 à 16 hr de subsistance sur la variété résistante Onward (2N à 16N), comparativement à des témoins élevés continuellement sur les variétés sensible (P) ou résistante (N), Différences non significatives (NS) ou significative à cinq (*) ou un (**) p. 100.

Les résultats démontrent que le jeûne de même que la subsistance sur une variété résistante réduisent la croissance (figs. 1 et 2) et la reproduction (fig. 2). Cette réduction est en général proportionnelle à la durée des traitements et est plus accentuée chez les larves soumises au jeûne. L'analyse statistique par le test *t* de Student a établi des différences significatives au niveau de cinq et un p. 100 entre plusieurs traitements. Les aphides ont subi assez bien des périodes de jeûne quotidien allant jusqu'à 12 hr, mais celles de 16 hr ont occasionné une mortalité de 21 p. 100 (expérience 2). Cependant, dans tous les traitements, toutes les larves survivantes ont atteint le stade adulte dans le même temps, à quelques heures près. Dans l'expérience 3, un jeûne quotidien de 2, 4, et même 8 hr n'a pas eu d'effets significatifs sur la croissance et la reproduction comparativement au témoin P (fig. 2), mais 4 et 8 hr de jeûne dans les expériences 1 et 2 (fig. 1) ont diminué la croissance d'une façon significative.

Certaines périodes de jeûne ont produit sur la croissance et la reproduction des effets équivalents à certaines périodes de subsistance sur une variété résistante. Ainsi, les larves élevées continuellement sur les plants résistants Onward ou Melting Sugar ont atteint un poids moyen équivalent à celles qui jeûnèrent 10 à 12 hr par jour; celles qui y passèrent 8 à 14 hr par jour ont atteint un poids moyen équivalent à celles qui jeûnèrent 4 à 8 hr par jour (Tableau I). Des conclusions semblables peuvent être tirées des effets des traitements sur la reproduction (Tableau I). Les effets d'une variété résistante sur la croissance, le développement et la reproduction des aphides sont donc comparables à ceux d'un jeûne partiel ou d'une sous-alimentation. Cette conclusion corrobore des résultats antérieurs qui indiquèrent que les aphides sur les variétés résistantes s'alimentent à un taux réduit (AUCLAIR 1958b, 1959), et que celles-ci, par ailleurs, contiennent en quantité moindre certaines substances azotées nécessaires à la nutrition des aphides (MAL-

TABLEAU I

Effets équivalents sur la croissance et la reproduction du puceron du pois de certaines périodes de jeûne et de subsistance sur une variété résistante

Expériences	Périodes de jeûne ou de subsistance sur une variété résistante (1)	Poids corporel mg/puceron		Périodes de jeûne ou de subsistance sur une variété résistante(1) (théoriques(2) ou expérimentales)	Poids corporel réel ou calculé (2) mg/puceron
2	N	2.5	⟶ (4)	10J	2.6(2)
3	N	2.3	„	11J	2.3
1	M	2.1	„	12J	2.1
1	4J	2.6	„	12M	2.6
3	4J	3.2	„	8N et 12N	3.1—2.6(3)
2	8J	3.1	„	14N	3.1
3	8J	2.9	„	10N	2.9
	Larves/femelle/48 hr (reproduction)				Larves/femelles/48 hr (calculées)
3	N	10.3	⟶ (4)	11.5J	10.4
3	4J et 8J	16.7 et 15.7	„	6N	16.0

(1) Voir Figures 1 et 2, ou protocole expérimental des expériences 1, 2 et 3.
(2) Par exemple, 2.6 mg pour 10 J représente la moyenne des pesées pour 8J et 12J.
(3) Différence entre les pesées 8N et 12N non significatives.
(4) Les flèches indiquent l'équivalence entre deux traitements.

TAIS & AUCLAIR 1957; AUCLAIR *et al.* 1957). D'autre part, les résultats des expériences 1, 2 et 3 infirment l'hypothèse de la présence de substances nuisibles, voires toxiques, chez l'hôte résistant, puisque la croissance et la reproduction des pucerons s'alimentant 4, 8, 12 ou 16 hr par jour sur un tel hôte furent généralement supérieures à celles des pucerons qui jeûnèrent durant ces mêmes périodes (figs. 1 et 2).

Au cours de 48 hr pendant l'expérience 3, les pucerons qui jeûnèrent respectivement 8 et 12 hr par jour ont excrété au total un plus grand nombre de gouttelettes que les témoins, soit 531 et 514 comparativement à 437 (Tableau II). A la suite de chacune des périodes de jeûne (durant lesquelles l'excrétion diminua considérablement), la fréquence d'excrétion a été doublée ou presque triplée (Tableau II, nombres avec astérisques sous fr.) par rapport au témoin P. Cette fréquence accrue a été accompagnée par une légère diminution du volume moyen des gouttelettes (Tableau II, d.m.); cependant, le taux d'excrétion subséquent aux périodes de jeûne fut quand même significativement supérieur à celui des témoins, soit 53.7 (8J) et 53.9 (12J) microlitres pour les périodes A et C, comparativement à 33.3 microlitres pour le témoin (P) durant ces mêmes périodes (Tableau II). Les quotients des taux d'excrétion obtenus de $\frac{(A + C)_{8J}}{(A + C)_{P}}$ et $\frac{(A + C)_{12J}}{(A + C)_{P}}$ ont été identiques, soit 1.6. Des expériences antérieures (AUCLAIR 1959) ayant démontré que l'excrétion par les pucerons est proportionnelle à leur ingestion, le taux précité d'excrétion accrue indique donc que les pucerons affamés purent augmenter „à volonté" leur taux d'ingestion sur des plants sensibles. D'autre part, des expériences de KENNEDY & MITTLER (1953) et MITTLER (1957) semblèrent démontrer que l'ingestion chez le puceron du saule, *Tuberolachnus salignus* (Gmelin), serait régularisée principalement par la pression de turgescence de la plante-hôte. Mittler souligna que l'ingestion et l'excrétion chez ce puceron étaient dépendantes de cette pression, tandis que l'aspiration active de la sève par l'aphide devenait d'importance négligeable. Cependant, BANKS & NIXON (1958) ont rapporté que l'excrétion chez le puceron de la fève *Aphis fabae* Scop., était accrue lorsque celui-ci était visité par les fourmis. Ils en conclurent que le puceron de la fève pouvait régler „à volonté" son taux d'ingestion et d'excrétion indépendamment de la plante-hôte. Les conclusions de nos travaux corroborent celles de ces derniers auteurs lorsque le puceron du pois s'alimente sur un hôte sensible.

D'autres résultats (expérience 4) ont confirmé que lorsque les aphides à jeun étaient placés sur un hôte sensible, la fréquence d'excrétion était augmentée comparativement à celle des témoins (Tableau III, nombres avec astérisques sous P8J et P16J comparativement à P). Le taux d'excrétion (et indirectement d'ingestion) a aussi été augmenté, ayant été de 108.5 (P8J) et 52.5 (P16J) microlitres pour les trois périodes subséquentes aux jeûnes comparativement à 70.1 et 30.0 microlitres pour le témoin P durant les périodes correspondantes. Cependant, aucune augmentation dans la fréquence d'excrétion n'a été observée lorsque les pucerons à jeun furent replacés sur un hôte résistant (Tableau III, nombres avec astérisques sous N8J et N16J comparativement aux nombres correspondants sous N). Le taux d'excrétion en a même été diminué, ayant été de 23.4 (N8J) et 12.6 (N16J) microlitres pour les trois périodes subséquentes aux jeûnes, comparativement à 42.5 et 17.9 microlitres pour le témoin N durant les périodes correspondantes. Les quo-

TABLEAU II

Effets de la variété résistante Onward et de périodes de jeûne sur le taux (en microlitres) et la fréquence (fr., nombre de gouttelettes) d'excrétion, ainsi que sur le diamètre moyen (d.m., en millimètres) des taches de miellat produites par 40 pucerons du pois au cours de quatre périodes de 12 heures de l'expérience 3.

Périodes consécutives de 12 hr (séparées par 48 hr entre B et C)	Témoins Pucerons maintenus sur Perfection (P)			Pucerons maintenus sur Perfection sauf durant												Pucerons maintenus sur Onward (N)		
				8 hr sur Onward en B et D (8N)			8 hr de jeûne en B et D (8J)			12 hr sur Onward en B et D (12N)			12 hr de jeûne en B et D (12J)					
	taux	fr.	d.m.	taux	fr.	d.m.	taux	fr.	d.m.	taux	fr.	d.m.	taux	fr.	d.m.	taux	fr.	d.m.
A	11.3	77	2.6	8.5	74	2.3	15.2	146*	2.2	5.6	60	2.1	9.1	130*	1.9	2.9	31	2.1
B	13.7	119	2.3	5.9	63	2.1	4.4	74	1.8	7.5	90	2.0	1.3	36	1.5	6.4	77	2.0
C	22.0	116	2.8	28.1	148	2.8	38.5	262*	2.6	20.1	127	2.7	44.8	327*	2.5	6.9	47	2.6
D	27.5	125	2.9	8.9	65	2.5	2.9	49	1.8	9.6	70	2.5	1.5	21	1.9	16.0	109	2.6
A + C	33.3	193		36.6	222		53.7	408		25.7	187		53.9	457		9.8	78	
B + D	41.2	244		14.8	128		7.3	123		17.1	160		2.8	57		22.4	186	
Totaux	74.5	437		51.4	350		61.0	531		42.8	347		56.7	514		32.2	264	
Poids final, mg/puceron	3.12			3.14			2.90			2.63			2.04			2.31		

* Fréquence d'excrétion subséquente à un jeûne.

TABLEAU III

Effets de la variété résistante Onward et de périodes de jeûne sur le taux (en microlitre millimètres) et la densité optique (d.o.) des taches de miellat produites par 40 pucerons

Périodes consécutives de 8 et 16 hr	Pucerons maintenus* sur Perfection (P)				Pucerons maintenus sur Perfection, sauf 8 hr de jeûne en A, C et E (P8J)				16 hr de j en B, D e (P16J)		
	taux	fr.	d.m.	d.o.	taux	fr.	d.m.	d.o.	taux	fr.	d.
A	6.8	73	2.1	—	0.5	9	1.7	—	10.7	115*	2
B	14.1	112	2.4	67	15.9	153*	2.2	69	0.5	8	1
C	10.7	78	2.5	64	0.6	6	2.1	47	13.0	95*	2
D	27.0	184	2.6	—	44.3	233*	2.8	—	0.9	11	2
E	12.5	66	2.8	—	1.8	22	2.0	—	28.8	131*	2
F	29.0	132	2.9	—	48.3	306*	2.7	—	0.4	6	1
A + C + E	30.0	217			2.9	37			52.5	341	
B + D + F	70.1	428			108.5	692			1.8	25	
Totaux	100.1	645			111.4	729			54.3	366	
Poids final mg/ puceron	2.23				1.95				0.		

* Fréquence d'excrétion subséquente à un jeûne.

tients des taux d'excrétion obtenus de $\frac{(B + D + F)_{P8J}}{(B + D + F)_{P}}$ et $\frac{(A + C + E)_{P16J}}{(A + C + E)_{P}}$ ont été respectivement de 1.5 et 1.8, tandis que ceux obtenus de $\frac{(B + D + F)_{N8J}}{(B + D + F)_{N}}$ et $\frac{(A + C + E)_{N16J}}{(A + C + E)_{N}}$ n'ont été respectivement que de 0.6 et 0.7.

La diminution dans l'excrétion par les pucerons des lots N8J et N16J, subséquemment au jeûne, pourrait être attribuable à un effet nocif et cumulatif résultant de leur jeûne et de leur alimentation intermittente sur la variété résistante. Cependant, les résultats d'une cinquième expérience n'ont démontré aucune diminution ni augmentation dans l'excrétion sur la variété résistante, subséquemment au jeûne et par rapport au témoin, lorsque les pucerons à jeun étaient placés *alternativement* sur un hôte résistant et sur un hôte sensible. Le taux et la fréquence d'excrétion pour l'ensemble de trois périodes de cette dernière expérience ont été respectivement 71.8 et 396 (N8J) comparativement à 72.2 et 484 pour N (Tableau IV), et les quotients des taux d'excrétion obtenus de $\frac{(B + D + F)_{P8J}}{(B + D + F)_{P}}$ et $\frac{(B + D + F)_{N8J}}{(B + D + F)_{N}}$ ont été respectivement 1.4 et 1.0. Les pucerons affamés placés alternativement sur la variété sensible et sur la variété résistante ne purent

ce (fr., nombre de gouttelettes) d'excrétion, ainsi que sur le diamètre moyen (d.m., en
16 hr au cours de l'expérience 4

erons maintenus sur Onward (N)			Pucerons maintenus sur Onward, sauf durant								
			8 hr de jeûne en A, C et E (N8J)				16 hr de jeûne en B, D et F (N16J)				Périodes consécutives de 8 et 16 hr
fr.	d.m.	d.o.	taux	fr.	d.m.	d.o.	taux	fr.	d.m.	d.o.	
46	1.9	—	0.8	13	1.8	—	4.9	53*	2.1	—	A
136	2.0	44	5.1	44*	2.3	52	0.8	12	1.9	48	B
61	2.3	57	0.8	16	1.7	31	4.0	32*	2.4	76	C
152	2.3	—	10.1	80*	2.4	—	0.8	16	1.7	—	D
56	2.5	—	0.7	10	1.9	—	3.7	27*	2.5	—	E
100	2.5	—	8.2	65*	2.4	—	0.6	15	1.6	—	F
163			2.3	39			12.6	100			A + C + E
388			23.4	189			2.2	43			B + D + F
551			25.7	288			14.8	155			Totaux
1.45			1.21				0.68				Poids final mg/puceron

augmenter leur excrétion sur cette dernière (rapport de 1.0), mais purent l'augmenter sur la première (rapport de 1.4), comparativement aux témoins placés continuellement sur la variété résistante ou sur la variété sensible. Il semble donc que l'ingestion de la sève par le puceron du pois sur un hôte résistant serait régularisée principalement par l'hôte, peut-être par des mécanismes cellulaires faisant appel à la pression osmotique et de turgescence, tel que suggéré par KENNEDY & MITTLER (1953) chez le puceron du saule; il est possible aussi que l'ingestion soit ralentie en partie par des structures histologiques défavorables de l'hôte résistant, ou par la présence de substances inhibitrices à l'acte d'ingurgitation, ou par une carence partielle de substances nutritives excitant l'appétit. Des résultats expérimentaux déjà rapportés (AUCLAIR 1958b) semblent appuyer la première et la dernière hypothèse.

Des travaux antérieurs (AUCLAIR 1959) avaient indiqué que les taux d'ingestion et d'excrétion des aphides sur des hôtes résistants étaient significativement inférieurs à ceux des aphides sur des hôtes sensibles. Les résultats (Tableaux II, III et IV) ont confirmé que ces taux étaient plus élevés chez les témoins P que chez N, et que l'excrétion d'acides aminés était plus abondante sur l'hôte sensible (Tableaux III et IV, densité optique des taches de miellat plus élevée chez P que N).

L'interprétation de BANKS & NIXON (1958, p. 710) concernant le mécanisme d'ingestion de la sève chez le puceron de la fève semblerait s'appliquer au puceron

TABLEAU IV

Effets de la variété résistante Onward et de périodes de jeûne sur le taux (en microlitres) et la fréquence (fr., nombre de gouttelettes) d'excrétion, ainsi que sur le diamètre moyen (d.m., en millimètres) et la densité optique (d.o.) des taches de miellat produites par 80 pucerons du pois par 8 et 16 hr au cours de l'expérience 5.

Periodes consécutives de 8 et 16 hr	Pucerons maintenus sur								Pucerons placés alternativement sur Perfection et Onward, mais jeûnant 8 hr durant les périodes A, C et E							
	Perfection (P)				Onward (N)				(P8J)				(N8J)			
	taux	fr.	d.m.	d.o.	taux	fr.	d.m.	d.o.	taux	fr.	d.m.	d.o.	taux	fr.	d.m.	d.o.
A	10.6	84	2.4	—	6.0	52	2.3	—	1.1	15	1.9	—	2.2	26	2.0	—
B	39.5	250	2.7	110	16.9	134	2.4	72	47.0*	320*	2.6	88	17.1*	108*	2.7	66
C	22.9	156	2.6	—	7.6	60	2.4	—	4.5	48	2.1	—	5.1	55	2.1	—
D	62.7	330	2.8	47	18.3	116	2.7	23	100.9*	531*	2.8	42	24.9*	131*	2.8	27
E	37.0	234	2.7	—	14.1	96	2.6	—	2.7	29	2.1	—	5.5	53	2.2	—
F	89.8	408	2.9	—	37.0	234	2.7	—	115.5*	608*	2.8	—	29.8*	157*	2.8	—
A + C + E	70.5	474			27.7	208			8.3	92			12.8	134		
B + D + F	192.0	988			72.2	484			263.4	1459			71.8	396		
Totaux	262.5	1462			99.9	692			271.7	1551			84.6	530		
Poids final mg/puceron	2.83				1.78				2.01				1.82			

* Taux ou fréquence d'excrétion subséquents à un jeûne.

du pois se nourrissant sur un hôte sensible. L'ingestion serait régularisée par la pompe aspirante de l'insecte. On présume que cette pompe serait normalement fermée, s'ouvrirait périodiquement pour admettre la sève et se refermerait ventralement afin de comprimer celle-ci vers l'estomac. Durant l'ingestion normale, la pompe s'ouvrirait et se refermerait à intervalles réguliers; lorsque les pucerons seraient affamés ou seraient visités par les fourmis, la pompe fonctionnerait à un rythme accéléré, introduisant ainsi la sève plus fréquemment dans l'estomac. La fréquence d'excrétion en serait ainsi accrue. Ces auteurs en concluèrent aussi que l'ingestion chez des aphides normaux se faisait apparemment de façon intermittente. AUCLAIR (1959) a de même rapporté que l'excrétion chez des pucerons isolés s'effectuait par cycles de quelques heures séparés par des périodes de non-excrétion.

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SUMMARY

*COMPARATIVE EFFECTS OF FASTING PERIODS, AND OF REARING PERIODS ON RESISTANT AND SUSCEPTIBLE PEA VARIETIES (*PISUM SATIVUM *L.), ON THE GROWTH, REPRODUCTION AND EXCRETION OF THE PEA APHID,* ACYRTHOSIPHON PISUM *(HARR.)*

Growth of the pea aphid, *Acyrthosiphon pisum*, reared continuously on resistant varieties Melting Sugar and Onward was comparable to that of aphids starved 10 to 12 hr daily. Growth of aphids reared on these varieties 8 to 14 hr daily was comparable to that of aphids starved 4 to 8 hr daily. Aphids not reared continuously on the resistant varieties spent the remaining period of each day on the susceptible variety Perfection. Similar results were obtained when reproduction was measured. Aphid growth and reproduction on resistant hosts were therefore similar to that of aphids subjected to semistarvation. These results do not support the hypothesis that substances toxic to the aphid are present in the resistant host.

Following each period of fasting, the frequency and rate of excretion (and ingestion) of aphids placed on a susceptible host were more than doubled, as compared to checks. However, no such increases in excretion were observed when fasted aphids were placed on a resistant host. These results suggest that sap ingestion can be increased when the starved aphid resumes feeding on a susceptible host, but cannot be increased when it resumes feeding on a resistant one. The hypothesis of BANKS & NIXON (1958) on the mechanism of sap ingestion by the bean aphid is discussed and appears applicable to the pea aphid feeding on a susceptible host.

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STUDIES ON WING POLYMORPHISM IN APHIDS

I. THE DEVELOPMENTAL PROCESS INVOLVED IN THE PRODUCTION OF THE DIFFERENT FORMS

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A theory of the nature of the developmental process resulting in wing polymorphism in aphids is proposed. It is suggested that all aphids begin development as presumptive alatae, and that apterae and intermediates are forms which have become irreversibly diverted from the alate course during development. Experiments are described which indicate that diversion resulting in the production of apterae can occur at any time over a protracted period extending from the late embryonic stages until the second larval moult. Prenatal diversion was demonstrated in the progeny of apterous aphids which had been kept on seedlings at 30° for a few days, and in the progeny of young alate aphids. Decapitation of adult aphids released the larviposition response and also resulted in prenatal diversion of their progeny. Postnatal diversion was readily brought about in first and second instar larvae by keeping them on host seedlings. In the third and fourth instars it could only be produced by extreme measures such as inflicting severe injuries on them, and by parasitization. It is suggested that the theory of polymorphism developed in this paper may be applied to other groups of insects with different forms of adults.

Although the factors influencing the production of alate and apterous aphids have been studied by a number of workers, the subject is still very imperfectly understood. The literature has been recently reviewed by BONNEMAISON (1951). A number of different factors have been claimed to be involved. They include temperature, humidity, photoperiod, nutrition, turgor of host plants, crowding, and the form, age and generation of the parents. Different workers have found different conditions to be effective, their results sometimes being contradictory. From the published work on the subject it is apparent that either different species and races of aphids differ considerably in regard to the factors influencing their form, or else techniques or rearing conditions employed by different workers have varied in some significant respect which has not hitherto been appreciated due to an inadequate understanding of the way in which the different forms are produced.

In the present paper a theory of the developmental process resulting in wing polymorphism in aphids is proposed. The theory owes much to previous authors, especially KLODNITSKY (1912), DAVIDSON (1927, 1929), and SHULL (1938, 1940), but it incorporates more recent work and provides a key for an explanation of some of the discrepancies which have appeared in the literature and a basis for future work on the factors affecting the production of the different forms.

No attempt will be made here to analyse the factors involved; this will form the subject of a further paper.

MATERIALS AND METHODS

The aphids used in this study were alienicolae of *Aphis craccivora* Koch which were reared on broadbean, *Vicia faba* L., variety Longpod. In South Australia *A. craccivora* overwinters in the parthenogenetic form and no gamic forms have been found either in the field or in laboratory cultures throughout this investigation.

Two of the principal exogenous factors influencing wing production in *A. craccivora* are the condition of its host plant and temperature. At a temperature of 30° C young bean seedlings less than 10 cm high favour the production of apterae, whereas at 20° C mature leaves of older plants favour the production of alatae. In the present study, the mature leaves were taken from halfway up the stem of plants about 1 m high. They were detached from the plants and kept in jars of water.

In some experiments leaf discs were used. Discs 1.5 cm diameter were cut from mature bean leaves and floated on water. Provided the leaf discs received adequate light and did not have too many aphids feeding on them they survived for several days and aphids could be reared on them from birth to maturity. They favoured alate production.

Early stages of the differentiation of wing anlagen were studied in histological sections of embryos and young larvae. The embryos were dissected out of the parent aphids and fixed and sectioned individually. All material was fixed in Bouin's fixative, sectioned at 5 μ and stained with Ehrlich's haematoxylin and eosin.

DIRECTION OF CHANGE OF FORM

It is generally considered that the more primitive phylogenetic condition of aphids was winged and that the wingless condition was a secondary development (TURNER & BAKER, 1916; DAVIDSON, 1927). This theory received support from the work of SHULL (1938) who found wing anlagen present in histological sections of all embryos and first instar larvae of *Macrosiphum euphorbiae* Thomas whether they were destined to develop into alatae or apterae. KITZMILLER (1951) found wing rudiments in some embryos of *Macrosiphoniella sanborni* (Gillette), not in others; and WHITE (1946) does not appear to have found them in any embryos of what may have been a different strain of the same species of aphid. Neither of these authors stated explicitly that the wing rudiments were totally absent; and Kitzmiller judged them to be present only when they were large enough to extend over several successive sections.

Studies of intermediates have been made by a number of authors in an attempt to obtain an understanding of the mechanism underlying the production of the different forms. DAVIDSON (1927) concluded that intermediates were forms which were tending to the apterous condition but in which the reaction was incomplete. KLODNITSKY (1912), ACKERMAN (1926) and RIVNAY (1937) recognized that intermediates were originally alatiform aphids in which a change of conditions during development resulted in the inhibition of the complete development of alatiform structures.

Despite the phylogenetic and histological evidence that the primitive condition of aphids is winged, and the evidence from studies of intermediates, most authors who have studied wing polymorphism in aphids have stated or implied a belief that the direction of change in form of developing aphids is from apterous to alate; that is, that the aphid proceeds to develop into an apterous individual unless some stimulus during development leads to wing production.

DAVIDSON (1927), on the other hand, proposed that the question which should be asked was not "what factors influence the appearance of alatae", but rather "what factors influence the appearance of apterae?" SHULL (1940) believed that the general trend of change was from alate to apterous, although he thought that temporary fluctuations in the course of development could occur, that is that change could take place in either direction. The reasoning which led him to believe that fluctuations were possible was based on the results of a study of the 'time of determination' theory by STILES (1938) which have since been shown to be untenable (JOHNSON, 1959c). RIVNAY (1937) found that wing development could not be induced by keeping aphids under alate-favouring conditions if they had been kept initially under apterous-favouring conditions; although it could still be interrupted in aphids which had begun their development under alate-favouring conditions, by exposing them later to apterous-favouring conditions. This suggested that the direction of change was from alate to apterous, and that it was irreversible.

We have examined serial sections of large numbers of fully-formed embryos and first instar larvae of *A. craccivora,* and have found distinct wing anlagen in all of them irrespective of whether or not they were destined to develop into alatae. The hypodermal thickenings first become apparent shortly before the embryonic moult (JOHNSON, 1958) which takes place about one day before birth; in aphids which proceed to develop wings, they increase in size until the embryo is born. Further growth and differentiation of the wing anlagen is discontinuous and occurs at each moult. During the first and second instars the wing anlagen are present as hypodermal thickenings and can be detected only in histological sections, in later instars external cuticular wingpads are present. In aphids destined to develop into apterae, wing anlagen are present at birth but they become reduced in size and disappear during the subsequent moults.

It is suggested that all virginoparae of *A. craccivora* begin their development as presumptive alates, and that apterae are aphids which have become diverted from the alate course during development. It will be shown that this diversion can occur over a protracted period during the late embryonic and early larval life of the aphid. Once aphids have been diverted from the alate course they do not appear to be able to revert to it. In the following experiments, except when they were exposed to the conditions which resulted in diversion, the aphids were reared on mature bean leaves which, under the experimental conditions, strongly favoured alate development.

DIVERSION FROM THE ALATE COURSE OF DEVELOPMENT

Pre-natal diversion

1. *Progeny of alate parents*

It has long been recognized that alate aphids have a marked tendency to produce more apterous than alate offspring and many authors have remarked on this (see Bonnemaison, 1951). Shull (1938) found that the wing anlagen of embryos and newly born larvae from alate parents of *M. euphorbiae* which were producing apterous progeny, were smaller than the wing anlagen of the progeny of apterous parents producing alate offspring. From a study of serial sections of embryos at various stages of differentiation, he concluded that the difference in size of the wing anlagen first became apparent about 24 hours before birth. He did not give any measurements of the thickness of the wing anlagen of the different forms of aphids, stating only that they could easily be distinguished from one another.

In *A. craccivora* we have found that the proportions of alate and apterous offspring produced by alate parents varies with the age of the parents. In Table I the results are given of an experiment in which the progeny of a batch of alate parents was reared to maturity on leaf discs. The alatae were collected from the roof of a rearing cage in which they had reached maturity. They were given a light knock-down dose of CO_2 to produce the settling response and then put on individual leaf discs which were kept in a glasshouse with an average temperature of 16° C. The alate parents were transferred at intervals to fresh discs and the larvae produced were reared to the fourth instar on the discs on which they were born, and their form was then assessed.

TABLE I

Form of the progeny of alate aphids when kept on leaf discs

Days from settling	No. of parents	No. of progeny	% apterae
1—3	28	368	97
4—7	28	307	45
8—11	28	208	34
12—15	27	102	31

The larvae born on the first three days nearly all developed into apterae, a high percentage of those born over the following days developed into alatae.

Confirmation that the larvae which were born in the first few days were diverted from the alate course of development before birth was obtained from histological studies. In Table II measurements are given of the maximum thickness of the wing anlagen of young first instar larvae from young alate parents and from apterous parents which were producing alatiform progeny. The wing anlagen of the larvae from young alate parents, while undoubtedly present, were considerably smaller than those of the larvae from the apterous parents.

TABLE II

Maximum thickness of wing anlagen of first instar larvae

Source of larvae	No. of wing anlagen measured	Thickness of wing anlagen in μ
Apteriform larvae — progeny of young alate aphids	16	6.6 ± 0.69
Alatiform larvae — progeny of apterous aphids . .	18	15.0 ± 1.02

Sections of embryos were also compared, and it appeared that the wing rudiments in embryos from young alatae ceased to develop at about the time of the embryonic moult, while the wing rudiments in embryos from apterae continued to grow until the embryos were born. Several first instar larvae from old alate parents were sectioned and were found to have large wing anlagen comparable in size to those of the progeny of apterous parents.

From these observations it appears that the first progeny produced by young alate parents of *A. craccivora* are mainly apteriform larvae which have been diverted from the alate course of development before birth. Within a few days of settling down on a host plant and beginning to reproduce alate aphids appear to be able to produce a high proportion of alatiform progeny which at birth have well developed wing anlagen. The change to alate production appeared to take place at about the same time as the general change in metabolism of alate aphids involving flight muscle histolysis and fat body hypertrophy (JOHNSON, 1959c).

2. *Progeny of apterous parents*

SHULL (1938) and KITZMILLER (1951) found that when apterae of *Macrosiphum euphorbiae* and *Macrosiphonella sanborni* respectively were transferred from conditions which favoured the production of apterae to conditions which favoured the production of alatae, there was a lag of up to three days before alate offspring were produced. They interpreted this as indicating that determination of the form of the progeny of apterous parents occurred before birth.

In *A. craccivora* we have similarly found that apterous aphids can be caused to produce either alate or apterous offspring according to the conditions to which they have previously been subjected. This is shown in the following experiment. Apterae from the same source were kept on fifteen bean seedlings, five of which were kept at each of 20° C, 25 ° C and 30° C for four days. The aphids were then placed on individual bean leaf discs in a glasshouse at an average temperature of 16° C where they were left to reproduce for two days. They were then removed and the progeny were reared to the 4th instar when they were counted and their form assessed. The results of the experiment are given in Table III.

TABLE III

Form of the progeny of apterae born within 48 hours after transfer of the parents from various treatments

Conditioning treatment of Parents	No. of Parents	Total No. of Progeny	% Apterae
4 days at 20° C on seedlings	18	130	21
4 days at 25° C on seedlings	18	105	26
4 days at 30° C on seedlings	18	116	83

The aphids which had been kept on seedlings at 30° C for four days produced mainly apterous offspring, whereas those kept at 20° C and 25° C produced mainly alate offspring.

Several first instar larvae produced by the females kept at 30° C were sectioned and were found to have large wing anlagen comparable in size to those of larvae destined to develop into alatae, suggesting that they had been diverted from the alate course of development later than the first progeny of young alatae.

3. *Decapitation of apterous parents*

When some insects are decapitated, a nervous inhibition of the oviposition response is removed and the headless insects go on to deposit eggs. When adult aphids are decapitated the larviposition response is released and the aphids go on to deposit all the fully formed embryos in their ovarioles.

Decapitation of aphids has been found to have an influence on the form of their progeny. A number of apterae of *A. craccivora* which had spent several days on mature plants at 15—20° C were decapitated and then put on five mature bean leaves where they reproduced. A control series of intact aphids from the same source was kept on another five leaves under identical conditions. All the parent aphids were removed from the leaves after 24 hours, and the progeny were reared to maturity before their form was assessed. The results are given in Table IV. The progeny of the decapitated aphids nearly all developed into apterae whereas the progeny of the intact aphids nearly all developed into alatae. The progeny of the decapitated aphids had apparently been diverted from the alate course as a result of decapitation of the parents.

It was thought that this diversion may have been a result of interfering with access of nutrients to the embryos, in that nutrient uptake by the parents was prevented by decapitation. To test this, the stylets were amputated from a number of adult aphids which were then released on bean leaves. Amputation of the stylets resulted in the aphids showing the settling response and they all settled down and reproduced on bean leaves just as the intact and decapitated aphids. The progeny of these aphids nearly all developed into alatae (Table IV).

TABLE IV

Form of the progeny of apterae which had been operated on in various ways

Replicate (each of 20 parents)	Intact Controls		Parents decapitated		Parents with stylets cut off	
	No. of progeny	% alatae	No. of progeny	% alatae	No. of progeny	% alatae
A	88	83	79	4	112	85
B	84	92	78	3	124	95
C	111	77	84	4	137	93
D	160	79	88	5	158	91
E	103	89	74	7	72	99
Average		84.0		4.6		92.6

Serial sections were made of the progeny of decapitated aphids and it was found that at least in larvae produced in the first day after decapitation of the parent, the wing anlagen were comparable in size to those of normal alatiform larvae but they broke down and disappeared almost entirely at the first moult. Some larvae born two days after decapitation had smaller wing anlagen but as they were also considerably smaller in overall size it is possible that they may have been born prematurely as a result of decapitation of the parent.

The switch-over of decapitated parents to the production of apteriform progeny was remarkably rapid: within 4 hours of decapitation, 227 larvae had been born and almost all of them developed into apterae. Both this, and the histological evidence that the wing-anlagen of these larvae were of the alatiform type at the time of birth, suggest that diversion from the alate course of development occurred well after the time it normally occurs in young alate parents, and either just before or at the time of birth. It is not known how decapitation of the parent brings about this effect. It does not appear to be the result of injury alone as severe cuts and burns to the thorax and abdomen of apterous aphids did not result in the production of apteriform progeny.

Post-natal diversion

1. *First and second instars*

Several authors including NEILS (1912), SHINJI (1918), WADLEY (1923), RIVNAY (1937) and BONNEMAISON (1951) have shown that in several species of aphids the proportions of larvae which developed into alatae and apterae could be varied by rearing them under different conditions during the first and second instars.

SHULL (1928) was unable to influence the form of larvae of *Macrosiphum euphorbiae* once they had been born and concluded that in that species the form of the progeny could only be influenced before birth. However, in his experiments, Shull took larvae of parents which had been kept under apterous-favouring conditions and exposed them to alate-favouring conditions. That is, he tried to induce wing development after it had already been interrupted. Had he attempted to suppress wing development in alatiform larvae he might have been able to demonstrate a post-natal effect. A further criticism of Shull's work has been made by BONNEMAISON (1951) and LEES (1959) on the grounds that he was probably producing gynoparae and not alate virginoparae.

TABLE V

Form of aphids which developed when alatiform larvae were reared on seedlings for the first or second instars and on mature leaves for the remainder of their development

Expt. No.	Period on seedlings	Total No. of aphids	Form (expressed as %)		
			Alatae	Intermediates	Apterae
1	First instar only	49	47	12	41
	Second instar only	54	44	30	26
	Controls (on leaves throughout)	46	85	6	9
2	Second instar only	150	35	29	36
	Controls (on leaves throughout)	358	94	0	6

In *A. craccivora* we have found that the form of larvae which have not already been diverted from the alate course can be influenced by the conditions to which they are exposed during the early instars. In Table V the results are given of experiments in which the progeny of apterous parents were kept on bean seedlings during the first and second instars and on bean leaves for the remainder of their development.

The larvae were born on bean leaves and were transferred within a few hours of being born or of moulting to the leaves and seedlings used in the experiments.

Larger numbers of apterae developed among the larvae which spent either of the first two instars on seedlings than among those which passed their whole development on mature leaves. A number of intermediates were also produced by the treatments. Most of them had very small wing rudiments.

It was not possible in this way to inhibit the differentiation of alatiform structures after the larvae had moulted into the third instar, and alatiform larvae which were transferred to bean seedlings as they moulted into the third instar continued to develop into normal alatae.

Similar results to these have been obtained by rearing larvae on mature bean leaves throughout development, but allowing ants to tend them for the first and second instars instead of transferring them to seedlings (JOHNSON, 1959b).

Third and Fourth Instars

Severe physical injuries inflicted on the abdomen of third instar alatiform larvae can cause them to develop into intermediates between alatae and apterae (JOHNSON, 1959a). The effect of the injuries was partly to inhibit the differentiation of alatiform structures, although the hypodermis in the vicinity of the structures was not itself injured. It was not possible to produce a similar effect by injuring fourth instar larvae.

Parasitization of alatiform larvae of different species of aphids by a chalcid wasp (TAKAHASHI, 1923), a cecidomyid fly (KIRKPATRICK, 1955), and a braconid wasp (JOHNSON, 1959a) has been found to result in suppression of the differentiation of alatiform structures. When first and second instar larvae of *A. craccivora* were parasitized by *Aphidius platensis* Brèthes (JOHNSON, 1959a) alatiform structures were completely or almost completely suppressed; when later instar larvae were parasitized, they retained their wingpads but these differentiated very little further and the aphids became intermediates with wing rudiments of various sizes according to their stage of development when the aphids were parasitized and whether one or more than one parasite egg was deposited in them.

DISCUSSION

The possible courses of development of alienicolae of *A. craccivora* from the late embryo until the adult stage are shown diagrammatically in Fig. 1. The aphids are shown to begin their development as presumptive alates. They can be diverted from the alate course before or after birth and once diverted they cannot revert to it. Diversion can be complete or incomplete. If it is complete and occurs before the beginning of the second larval moult the aphids develop into apterae; if it occurs later they become intermediates. Incomplete diversion results in the aphids developing along a course between the extremes and becoming intermediates. All changes in the course of development are away from the alate course.

The earliest instance that has been found of diversion from the alate course of development in *A. craccivora* is in the progeny of young alate parents. Histological evidence suggests that diversion in these embryos occurs at about the time of the embryonic moult. WHITE (1946) and KITZMILLER (1951) who were unable to find conspicuous wing anlagen in some embryos of apterous parents of *M. sanborni* may have been dealing with another instance of diversion at about the

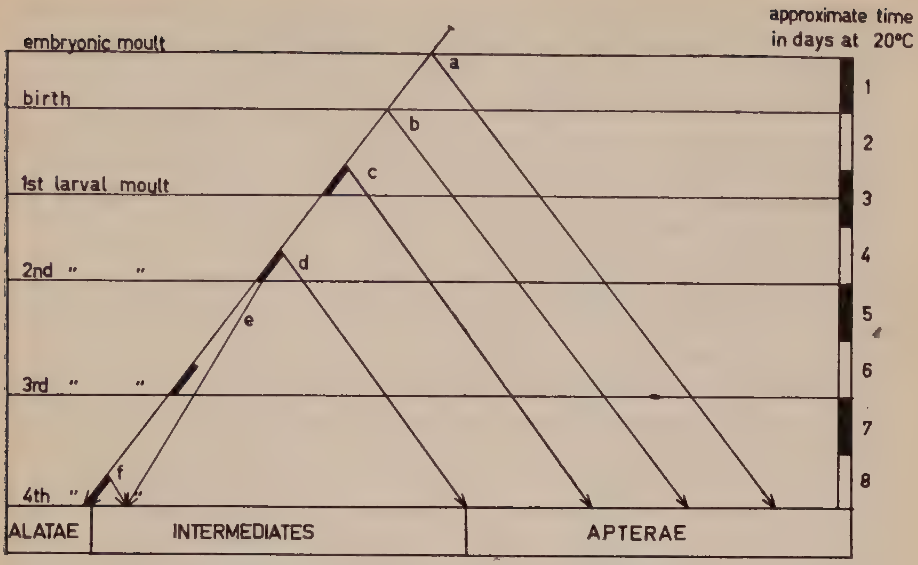

Fig. 1. Diagram showing possible courses of development of virginoparae of *Aphis craccivora* Koch. Demonstrated times of diversion from the alate course are indicated. (a) Progeny of young alate aphids; (b) Progeny of decapitated parents; (c) Postnatal diversion in first instar larvae; (d) Postnatal diversion in second instar larvae, the last time at which diversion can result in the production of normal apterae; (e) Incomplete diversion in second instar resulting in an intermediate; (f) Extreme diversion in fourth instar caused by parasitization and producing an intermediate with the same general characters as (e). Heavy lines indicate the approximate duration of each moult. In aphids which become alatae, the last instar typically lasts one day longer than is indicated.

same time but in apterous parents, but from the limited data they give no definite conclusions can be drawn. In *A. craccivora* all the progeny of apterous aphids that were sectioned had larger wing anlagen than the progeny of young alatae, whether or not pre-natal divergence was taking place. This suggests that diversion occurred at a later time during their embryonic development than it occurred in the embryos of young alatae. Nothing is known at present of the means by which the parent aphid influences the form of its progeny.

It has recently been suggested (KENNEDY & STROYAN, 1959; JOHNSON, 1959a) that the differentiation of alatiform structures in aphid larvae may be controlled by the same endocrine system as controls metamorphosis. If this is so the theory of form reversal outlined above could be summarized as follows: originally the endocrine system of the insect, or its controlling mechanism, is "set" to favour the differentiation of alatiform structures. During development in response to various stimuli it can be "reset" in such a way that the differentiation of alatiform structures is inhibited. It sometimes happens that when it is "reset", the modification is incomplete and intermediates result.

It is suggested that the term "wing determination" which has commonly been used in studies on the production of winged and wingless forms of aphids is misleading. It is generally interpreted as meaning determination of the insect as a whole to develop into an alate individual, but as we have shown in *A. craccivora* and as can be inferred for other species, alate aphids are not produced by a process of determination. That is, they do not pass through a limited period of development during which they are competent to respond to stimuli by making a permanent "decision" to develop into alatae. On the contrary, they begin development as presumptive alatae and can be diverted from this course to become apterae. Wing polymorphism in other insects such as leafhoppers, thrips, Orthoptera, etc. is probably similar in character to that in aphids and it is suggested that here too it is wrong to consider it in terms of "wing determination" as this implies a change in the opposite direction.

In studies on the production of sexual forms of aphids most workers have considered the subject from the point of view of factors causing the production of the sexual rather than the parthenogenetic forms. However, there can be little doubt that phylogenetically in female aphids the sexual is more primitive than the parthenogenetic form, and just as developmentally apterae result from deviation from the more primitive alate course, so parthenogenetic females result from deviation from the more primitive sexual course of development. This is clearly brought out in the experiments of LEES (1959) who found an "asymmetry" of the response of embryos of *Megoura viciae* Buckton when the photoperiodic regime was changed during their development from short to long and vice versa. His results accord well with the theory that embryos begin development as presumptive oviparae and can be irreversibly diverted from this course at any time until they have reached a certain size. From Lees' data, embryos appear to remain

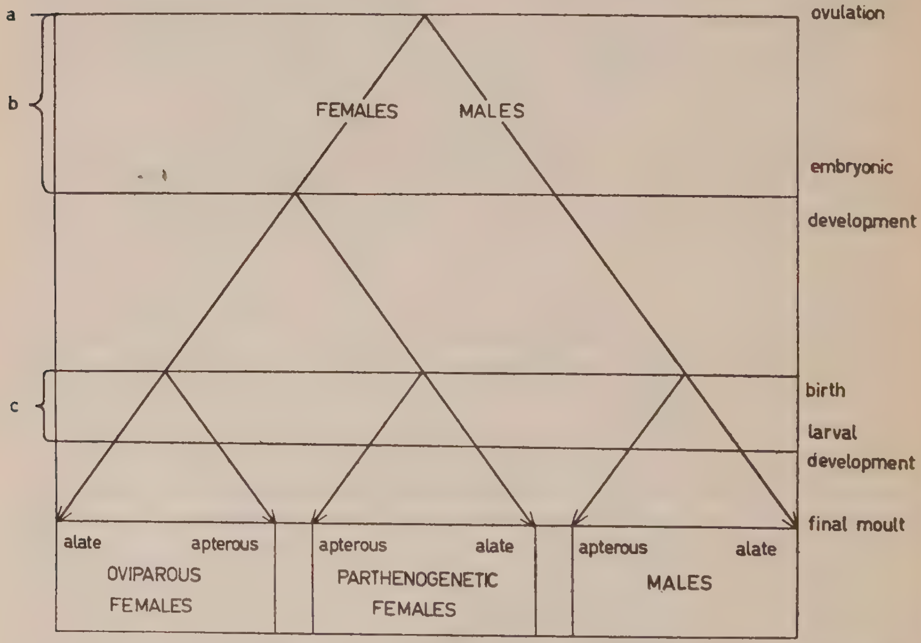

Fig. 2. Suggested developmental relationships between the common forms of aphids. The intervals indicated on the right of the diagram are not to scale; a) determination of sex; b) period during which female embryos can be diverted to the parthenogenetic course; c) period during which embryos and larvae can be diverted from the alate to apterous course.

competent to be diverted to become parthenogenetic until they are between the sizes of the third and fourth embryos in the ovarioles of larvae and teneral apterae as shown in his figs. 3 and 4. To become oviparae the embryos must pass all their development from ovulation until this stage under a short photoperiodic regime.

The developmental relationships between the different common forms of aphids are shown diagrammatically in Fig. 2. The first division (a) involves the determination of sex. Then accepting the phylogenetic argument that the primitive condition of aphids is winged, the normal courses of development from the determination of sex would be towards the alate male and the alate oviparous female. During early embryonic development female embryos can be diverted from the more primitive sexual course to become parthenogenetic (b). A further diversion (c) may occur in each of the resulting developmental courses, and this results in the suppression of wings and the consequent development of apterous forms of males and parthenogenetic and oviparous females. In most species of aphids, the condition of the sexual forms with respect to wing development has become fixed and the males and oviparous females that occur within a species are usually all either winged or wingless; typically males are winged and females are wingless. The period of competence of aphids to be diverted from the alate to apterous course of development, i.e. between the embryonic moult and the second larval moult, appears to coincide with the period of competence of the first embryos in their ovarioles to be diverted from the oviparous to the parthenogenetic course of development as indicated by LEES' (1959) results. This suggests that in species of aphids with host alternation, this is also the time when the decision is made whether the larvae will develop into alienicolae or sexuparae.

Polymorphism in other groups of insects

The theory outlined above for the development of the different forms of aphids may also apply to caste production in social insects. It is recognized in termites (WEESNER, 1952) that all individuals begin development with an "initial trend" towards the alate reproductive and that worker and soldier castes result when development along this course is inhibited. LUKOSCHUS (1956) considered that in bees the queen was the " normal" insect and that workers were a modification. In social insects generally, production of the various castes should perhaps be considered not as "caste determination" which may be misleading for much the same reasons as "wing determination" in aphids, but as diversion from a more primitive alate or reproductive course of development. It is possible that in some species, as in aphids, diversion may occur at any time over a considerable period, encompassing both embryonic and post-embryonic development. If this is found to be so, disagreements may be resolved that now exist regarding the time that the various castes of some social insects becomes fixed (BRIAN, 1957). The parallel between polymorphism in aphids and caste production in social insects is further underlined by the accumulating evidence in both fields that differentiation of the structural features which characterize the different morphological forms is under endocrine control (LUKOSCHUS, 1955; LÜSCHER, 1958; JOHNSON, 1959a).

In all the cases of polymorphism quoted above, diversion from the more primitive course of development has been towards a more specialized form lacking some of the characteristics of adult insects, either their specialized sensori-motor apparatus (aphids), or their ability to reproduce (worker castes of bees), or both of these (worker and soldier castes of ants and termites). Phase transformation in locusts is another instance of polymorphism and here diversion from a more primitive course of development also occurs, but it differs in that it is in the opposite direction, towards a "super-adult" (KENNEDY, 1956). In locusts the solitary form can probably be regarded phylogenetically and developmentally as the more primitive. In those species which exhibit phase characteristics, phase *gregaria* is a result of diversion from the more primitive *solitaria* condition, and, as with aphids, the stimulus resulting in diversion can be applied either to the embryo or the larva, but in locusts, unlike aphids, diversion is not an irreversible process (ELLIS, 1959).

ZUSAMMENFASSUNG

UNTERSUCHUNGEN ÜBER DEN FLÜGELPOLYMORPHISMUS BEI BLATTLÄUSEN. I. DER MIT DER ERZEUGUNG DER VERSCHIEDENEN FORMEN VERBUNDENE ENTWICKLUNGSVORGANG

Bei *Aphis craccivora* Koch wurden in älteren Embryonen und Larven des 1. Stadiums rudimentäre Flügelanlagen gefunden, gleichgültig ob sie zur Entwicklung von Geflügelten determiniert waren oder nicht.

Die erstgeborenen Embryonen junger geflügelter Mütter hatten bei der Geburt kleinere Flügelanlagen als die Embryonen ungeflügelter oder alter geflügelter Mütter, und sie entwickelten sich fast ausnahmslos zu Ungeflügelten.

Wenn flügellose Erwachsene für einige Tage 30° ausgesetzt wurden, erzeugten sie überwiegend flügellose Nachkommen; bei niedrigeren Temperaturen brachten sie hauptsächlich geflügelte Nachkommen hervor.

Dekapitation der Flügellosen führte zum Absetzen einer Anzahl von Larven, die sich fast alle zu Ungeflügelten entwickelten.

Larven des 1. und 2. Stadiums, welche sich zu Geflügelten entwickelt haben würden, wenn sie an reifen Blättern ihrer Wirtspflanzen gezüchtet worden wären, entwickelten sich zu Ungeflügelten oder Intermediärformen, wenn sie an Sämlingen gehalten wurden.

Larven des 3. and 4. Stadiums konnten von der Entwicklung zu Geflügelten nur durch so einschneidende Maßnahmen wie Parasitierung oder Zufügung ernster Verwundungen abgehalten werden und wurden dann zu Zwischenformen.

Es wird angenommen, daß alle Blattläuse ihre Entwicklung als präsumptive Geflügelte beginnen und daß sie während der Entwicklung vom späten Embryonalstadium bis zur zweiten Larvenhäutung jederzeit irreversibel von dieser Entwicklungsrichtung abgelenkt und zu Ungeflügelten umgestimmt werden können.

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